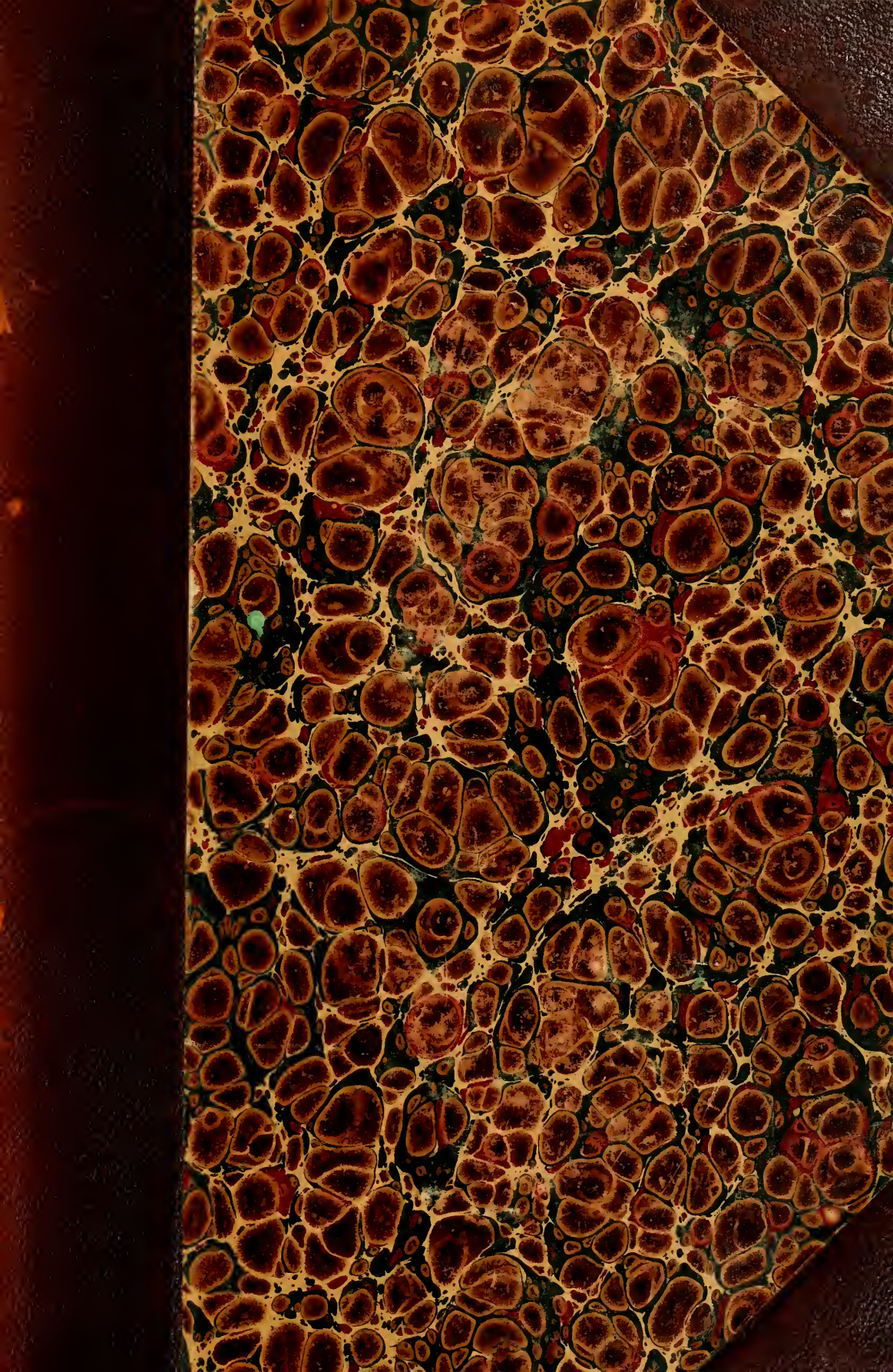

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FOUNDED BY C. O. WHITMAN

EDITED BY

J. S. KINGSLEY
University of Illinois
Urbana, Ill.

WITH THE COLLABORATION OF

GARY N. CALKINS
Columbia University

EDWIN G. CONKLIN
Princeton University

C. E. McCLUNG
University of Pennsylvania

W. M. WHEELER
Bussey Institution, Harvard University

WILLIAM PATTEN
Dartmouth College

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GERM CELLS OF COELENTERATES

V. EUDENDRIUM RAMOSUM

GEORGE T. HARGITT

From the Zoological Laboratory, of Syracuse University

NINETEEN FIGURES

1. INTRODUCTION

In this series of studies forms have been considered which showed some differences in origin, growth, and ripening of the germ cells, or which were taken as types of certain taxonomic groups. It has been found that the different types of coelenterates do not necessarily show distinctions with respect to their germ cells, but may, on the contrary, be essentially alike. The study of Eudendrium was undertaken, not as a type of some morphological group, but because of certain obvious, though more or less superficial, peculiarities in the history of its germ cells, and in particular with the egg cells.

Whatever may have been found to be the ultimate method and place of origin of the egg cells in most of the forms studied, a cursory examination would usually show the egg cells to be first observable in the reproductive bodies, whether they were gonophores or medusae. In Eudendrium this is not the case. No doubt most zoologists who have examined this hydroid, or have made use of it for class work, have noted the presence of egg cells in the stem or branches, even when there were no reproductive bodies in that neighborhood. Such a condition is a common and rather striking feature of Eudendrium and might indicate some peculiarity of the germ-cell history. It is also true that the history of the egg cells in Eudendrium has been discussed rather widely and certain phylogenetic implications made which have considerable theoretical importance. These considerations have led to a new study of the germ cells of this form, with a brief review of the work previously done.

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Eudendrium is a common Tubularian hydroid of our Atlantic coast; it is also widely distributed in other parts of the world. The reproductive cells are developed within simple, more or less sac-like, gonophores which in the mature animals seem to have few, if any, of the characteristic structural features of polyps or of medusae. However, such gonophores (often called sporosacs) are commonly referred to in discussions and in text-books as degenerate medusae or medusoid buds, and it is this assumption of the degenerate medusoid character of the gonophores which is mainly responsible for certain interpretations of the germ-cell history of Eudendrium which I believe are erroneous. It is only necessary to point out that the female gonophores, at least, arise from, and by the modification of, a hydranth or polyp which, in the beginning, resembles in structure other polyps of the colony. Since this may easily be demonstrated by a study of the developing reproductive bodies, it is manifestly quite incorrect to speak of the female gonophores of Eudendrium as medusoids, and any discussion, phylogenetic or otherwise, which proceeds upon such a basis is, of course, quite futile and meaningless. While I have not given the same attention to the method of formation of the male gonophores, it may be doubted whether these have any more of the medusoid character than do the female gonophores.

Ciamician ('78), studying Eudendrium ramosum, decided the egg cells arose from the ectoderm of the gonophore itself. His figures clearly show that he distinguished the egg cells only after they had become of some size, and his conclusions do not, therefore, touch the question of the real place and method of origin. Kleinenberg ('81) came to the conclusion that the egg cells arose in the ectoderm, passed into the entoderm, and migrated into the gonophore, where they again entered the ectoderm and completed their growth and development. Weismann ('83), in his study of E. racemosum, found egg cells in the ectoderm and entoderm, but, since those in the ectoderm were always smaller, concluded this layer was the place of origin. The region of the colony concerned in the formation of the eggs was the stem just below the terminal hydranths. He accounted for

this as the germinal zone, chiefly on account of the abundant food supply in a region so close to the polyps, thus calling attention to the essential relation between germ-cell formation and abundant nourishment. This region is also near the growing zone and the germ cells were believed to arise only from young cells. However, these young cells were regular ectoderm cells and not a special kind of cell, for he says:

> One can say positively, that the same small egg cells] are visibly distinguishable in no way from other young ectoderm cells, for one can always recognize the place at which later a germ zone will form and can demonstrate that in that place are no cells which could be so designated [as germ cells]. The germ cells are therefore not in this case definitely contained in the colony from the beginning and differentiated only at the time of sexual maturity, but they arise only at this time out of a growing mass of young cells.

This essential relation between the origin of germ cells and an abundant food supply so positively stated by Weismann and since then abundantly confirmed by other authors, he did not maintain in later publications. In like manner, his own observations and demonstrations upon the origin of the germ cells from the ordinary cells of the hydroid body, both of ectoderm and of entoderm, he later discarded in favor of a theoretical view that they came from a peculiar and self-perpetuating tissue or substance, the germ substance. Weismann studied other species of Eudendrium, notably E. capillare, in which he found egg cells only in the entoderm. Their origin and growth, he says, must therefore be from and in the entoderm alone, "since it is inconceivable, that otherwise one should never find egg cells in the ectoderm."

C. W. Hargitt ('04) studied several species of Eudendrium. He found the egg cells of E. racemosum both in ectoderm and entoderm, but believed the entoderm to be the place of origin, since it contained the smaller cells. The same was true for E. dispar and E. ramosum. Weismann's account for E. capillare was entirely confirmed, viz., that egg cells were found always and only in the entoderm, and a similar condition existed in E. tenue. This author finds that "the ova arise in hydroids by

differentiation of cells of either one or both the entoderm and ectoderm." Growth takes place in the position where they are formed through the nutritive activities of surrounding cells, and a migration toward the gonophore region is also characteristic. It is suggested that the presence of germ cells in a certain region may act as a stimulus to the production of gonophores. Goette ('07), studying E. rameum and E. racemosum, found the smallest germ cells in the ectoderm, and he looks upon this as the seat of formation of germ cells in Eudendrium. From his work on various hydroids he is convinced that the place of origin of germ cells in these organisms is a varied one in different species or even in the same species; i.e., the germ cells may sometimes arise in the ectoderm and sometimes from the entoderm, even in the same species.

In regard to the formation of male germ cells, Ciamician ('78) believes the sperm mother cells arise by transformation of entoderm cells within the gonophore. It may be doubted whether his conclusions can be considered really to touch the matter of ultimate origin; his figures do not give this impression. Weismann ('83) is of the opinion that they arise not in the gonophore, but in the stalk of the hydroid and later migrate into the gonophore. He confirms Ciamician on their entodermal origin. Goette ('07) sees the ectoderm as the point from which the sperm cells as well as the egg cells come.

These are the chief papers dealing with the facts of germ-cell formation in Eudendrium. Other papers are found which discuss the facts without adding any observations or new facts. Weismann ('04) discusses Eudendrium and its germ cells along with other hydroids and here takes the positive ground, contrary to his own earlier brilliant observations, that the germ cells of Eudendrium and of all Hydromedusae arise in the ectoderm. This is apparently a necessary assumption for him, since his thesis of the distinctness and self-perpetuation of the germinal substance requires more constancy of germ-cell position than the observed facts present. The same hypothesis also involves germ-cell formation early in the development of the hydroid and an unbroken line of such cells up to the time of the

formation of the reproductive bodies. This part of the theory is established by making certain ontogenetic and phylogenetic implications concerning the degeneracy of the gonophores and the origin of germ cells in the stem and not in the gonophore. These implications are not firmly grounded upon fact.

This later theoretical position of Weismann is the one which has been generally accepted and is the position almost universally taken in text- and reference books and in general papers not dealing specifically with the subject of germ cells of coelenterates.

2. OBSERVATIONS UPON EGG CELLS

Study of entire stems. Examination of a stained and cleared portion of a female colony, such as is shown in figure 1, usually shows a number of deeply staining cells, some large and others small, scattered through the stem. The large cells appear as growing eggs and the small ones seem to be like them except in size. The impression gained from the study of such a preparation is that of a considerable number of egg cells scattered through the colony and widely distributed along the stems of the hydroid.

A detailed and careful study of these cells was made and their distribution plotted. About sixty slides, made by staining and mounting entire portions of different colonies made up of stems, branches, polyps, and gonophores, were examined. These observations demonstrated that the deeply staining cells were not universally distributed, but were more or less localized in certain regions, especially in the branches which come from the main stem of the hydroids. Figure 1 is a camera outline of a part of a colony in which the dots mark the position and relative size of the deeply staining cells. In all such preparations the hydrorhiza and main central stem of the hydroid showed no deeply staining cells, the bases of the branches arising from the main stem were likewise devoid of such cells, but further distalwards they were present, sometimes in considerable numbers. The pedicels of young lateral hydranths and of gonophores, as well as the distal and younger parts of the lateral branches even where hydranths and gonophores were absent, were the regions

in which these cells were most abundant. The cells, of varying sizes, were usually in groups, the largest cells always opposite the point of emergence of a branch which bore small hydranths or gonophores, while in the stem both distalwards and proximalwards the cells become smaller. The base of each hydranth or gonophore pedicel seems to be the center of a group of these cells, a condition suggesting their local origin. Where no gonophores are present these deeply staining cells may still be grouped at different places, but the larger cells in the center of such groups may be demonstrated to be at about the place where gonophores develop as the colony grows. In most groups of this character the cells are quite small, no large growing eggs appearing till the gonophore begins its development. On the other hand, where gonophores are forming or have formed, the adjacent group of deeply staining cells show numerous large cells and few small ones. The size and location of the deeply staining cells lead one to look upon them as egg cells, but it is essential to secure positive evidence on this point. If these are egg cells it must be further determined whether they arise in these positions or have reached them by a migration from a more or less distant point of origin.

Figure 2 is an outline of an optical section of the part of the stem shown at *A* in figure 1. While the difference in size of the deeply staining cells may not be especially significant, their difference in position is material. Those cells which extend from the supporting layer to the enteric cavity are gland cells, the deeper cells which do not reach the enteric cavity are egg cells. Figure 3 represents *B* of figure 1 and shows a number of egg cells all in the process of growth. Figure 4 is a similar drawing of region *C* of figure 1. Here most of the deeply staining cells do not touch the enteric cavity; the larger ones show all the characteristics of egg cells without any question, most of the smaller ones are also egg cells. Figure 5 is an optical section of a distal portion of a stem of the colony in a region devoid of gonophores or lateral branches and hydranths. So far as the staining is concerned, they are quite like the cells shown in figures 2 to 4, but most of them extend the full distance from

the supporting layer to the enteric cavity. In some (*gl*) the cytoplasm bordering the cavity stains differently than the deeper part of the cell; these are gland cells. Other cells do not extend to the cavity, and still others, while they extend to the enteric cavity, do not show such a differential staining capacity. Some of these are gland cells, while the interpretation of the others is uncertain.

The study of entire stems in optical section is necessary and very helpful in determining the number, size, shape, and distribution of the egg cells, but lack of sharpness in such preparations, especially with high powers, makes it impracticable to secure positive evidence concerning the finer details of structure of the smaller cells. Such evidence must be gained from the study of sections; this will be described very shortly.

A second problem possible of elucidation by the study of entire stems concerns the migration of germ cells. Weismann ('83, '04) claims the egg cells arise only at the distal end of the main stem and at the distal ends of the chief branches, from which points there are extended migrations into the gonophores. I do not understand this to mean that after a colony has become mature the germ cells begin to form from the young cells at the distal ends of branches and migrate throughout the colony to the gonophores, which are especially abundant toward the base of the colony. Such a migration does not occur, for the main stem and the basal ends of the larger branches are always devoid of egg cells, which could hardly be the case if egg cells moved from the distal ends of branches to the lower branches. Weismann states in his earlier works that egg cells may only migrate distally, never proximally, though the contrary is implied in the figures and descriptions of his later discussions (Weismann, '04, vol. 1, p. 414). I take it, rather, that he means the germ cells arise in the young parts of the colony, migrate toward the regions where gonophores are to be produced and upon their appearance the egg cells take their position in the gonophores.

The migration just outlined would not be very great in extent, and it is probable that some such movement takes place. The egg cells on the right of figure 4 are elongated as though

killed in the midst of an amoeba-like action. The mode of formation of the gonophore, however, suggests more of a passive transportation of the egg cells due to the growth of the stem and gonophore; this would be sufficient to account for a part of the translocation of these cells. Goette ('07) has pointed out the probability of this as a factor in the location of germ cells within the gonophores of other hydroids, and it seems to be significant here. Figure 6, an optical section of a very young gonophore rudiment, shows the point clearly. The hydranth which becomes entirely transformed into several gonophores is almost sessile as it first arises, but ultimately it has a long stem or pedicel. The growing point is in the stem below the hydranth, and any germ cells which might have been in the young rudiment would be pushed far away from the main stem in the growth of the hydranth stalk. At *A* in figure 1 and in the hydranth between *A* and *B* we have the region of formation of future gonophores. Germ cells are already present here in the stem, and when the tissues of the stem begin to evaginate to form the gonophores, the germ cells present would be carried outward during growth. This would account in part, but probably not entirely, for the presence of germ cells in the gonophores.

Some indirect evidence also bears on this same question of migration. It is very common to find the conditions shown in figure 4, where a stem contains large and small germ cells side by side. If there be a definite germ region and a definite path of migration, a section of any portion of the stem should contain germ cells of about the same size; the presence of large and small egg cells in the same region, therefore, may be taken as evidence of the formation of the small cells in that position. If the evidence is accepted, the large cells may be considered to have arisen in that same location and their shape is not indicative of migration. Other evidence is at hand which seems to suggest the formation of egg cells at various places, a growth in those positions and little or no migration. Figure 1 shows groups of cells all along the branches, some large and others small, each group centering about a lateral hydranth or gonophore. Such a condition is more consistent with the origin of

each such group of egg cells in that place than with a single germinal zone at some distance and later migration of eggs into the places of ripening and development.

One other point of general interest comes from this study of entire stems or parts of colonies. At *A* in figure 1 there is a group of egg cells in the stem, the same is true in region *B*, and between the two is a hydranth whose stalk contains eggs. Gonophores are not present here, but these are regions where gonophores would have developed in the living colony. The evidence for this is easily obtained by a study of gonophore formation in different colonies. Probably the simplest explanation for such an arrangement is the suggestion of C. W. Hargitt ('04) that the presence of a group of egg cells may be the stimulus for the formation of the gonophore. So far as I have observed in Eudendrium ramosum, groups of germ cells are always present before a gonophore begins its development.

Study of sections. The study of stained and cleared portions of the colony gave a clear understanding of the relative position of egg cells in stems, hydranths, and gonophores, and of the numbers and sizes of egg cells, but did not permit a positive determination of the place and method of origin of such cells. It was also impossible to distinguish small egg cells and gland cells, since all deeply staining cells had much the same appearance. These difficulties should not obtain in sections, hence various parts of the colony were sectioned and studied.

A longitudinal section through the stem, just proximal to a terminal hydranth, is shown in figure 7, one-half the width of the section being represented. In the entoderm a typical egg cell which has undergone some growth is shown at *ov*, lying deep in the entoderm against the supporting layer and separated from the enteric cavity by entoderm cells. Its cytoplasm is composed of uniformly distributed granules, large and deeply stained; the nucleus is large, with a distinct nucleolus, and contains a relatively larger amount of chromatin than the regular entoderm cells. The chromatin is distributed chiefly about the nuclear membrane with a small amount forming a network in the nuclear sap. These characteristics are more or less applicable

to all egg cells until they become very large; the position of egg cells, deep in the tissues against the supporting layer, is also characteristic. Another egg cell, *ov*, quite similar, but staining less deeply, is present in the ectoderm. At *a* in the ectoderm are cells which differ in certain respects from other ectoderm cells, especially in a slightly more compact and deeper staining cytoplasm and in having a nucleus somewhat larger than nuclei of other ectoderm cells. If we may speak of these as primordial egg cells just arising from the interstitial cells of the ectoderm, it would suggest an ectodermal origin of egg cells in Eudendrium.

Figure 8—a continuation of the same stem shown in figure 7—shows at *ov* two small egg cells in the entoderm and none in the ectoderm. These show all the characteristics enumerated for the larger eggs just described. At various other places in the sections of the same stem similar cells are found, both in ectoderm and in entoderm. In the entoderm gland cells (fig. 7, *gl.*) might be mistaken for egg cells, but their shape and position differentiate them, even though the staining capacity is somewhat like that of egg cells. Many other cells from a similar portion of colonies, as well as from regions adjacent to hydranths and gonophores, showed these egg cells. The very large eggs were always in the entoderm, but as there were also small cells in the entoderm and some undoubted egg cells in the ectoderm which were larger than some in the entoderm, it is not possible merely on the basis of size to determine which layer is the seat of origin of the eggs.

Figure 9 is from a transverse section of a portion of a stem at the place of junction with the stalk of a hydranth and developing gonophores. In the entoderm is a very large growing egg and at *ov* in the ectoderm (lower right side of figure) is a small cell which has much the appearance of an oöcyte at the very beginning of its growth period. The deeply staining cytoplasm, the large, richly chromatic nucleus, the spireme or loop-like character of the chromatin, have every appearance of cells found in other coelenterates which were interpreted as some phase of synizesis or synapsis. This cell can hardly be an ectoderm cell in an ordinary division, since it is the only cell

in this region, either in ectoderm or entoderm, which has such an appearance; also it has certain obvious differences from all the adjacent ectoderm cells, and just as obvious resemblances to a young oöcyte. This cell is the only one found which presented such an appearance; neither in ectoderm nor entoderm of any of the material could a similar cell be discovered. One cell is not sufficient to warrant any conclusions with respect to the interpretation of this appearance, but in view of its similarity to such stages in Campanularia, Clava, Aglantha, and other coelenterates, it may possibly be of the same nature. A considerable amount of material was used, secured at slightly different times, and many sections made; just why the early stages so clearly demonstrated in other forms were so rare (or absent) in Eudendrium is not known. The material used showed in every case the beginning of gonophore formation, and it is possible this is too late to show the origin of egg cells, but from every colony younger portions devoid of gonophores were examined and one would expect the germ cells to be in an immature condition if they were present.

The place of origin of egg cells in this form cannot be positively determined from the material at hand, though I incline to the opinion that it is the ectoderm and that the small cell *ov* of figure 9 marks the earliest differentiation of such a cell. In this respect my observations confirm the belief of some workers and are opposed to those of others; but the place of origin is not, in my opinion, a matter of especial significance, and I agree with C. W. Hargitt ('04) and Goette ('07) that there may be variation in the place of formation of the germ cells in hydroids, perhaps even in the same species. The place of origin not having been demonstrated, the method of origin is likewise uncertain. In Campanularia and Clava the derivation of germ cells from the tissue cells was clear; the position of the smaller egg cells in Eudendrium resembles that of these other forms. Again one may have the suspicion that Eudendrium agrees in a general way with the method of germ-cell formation of those forms, but no more may be said on the evidence obtained from this study.

Growth of egg cells. Whatever may be the origin of the egg, it lies for the entire early period of its growth within the entoderm. An explanation for this is obvious, in such a position the egg is very close to the enteric canal with its circulating fluid from which the nutrient material is obtained for growth and for elaboration of reserve stuffs. Figures 3, 4, and 6 to 9, show the relation of growing eggs to the entoderm and enteric canal. Since the egg does not come into contact directly with the enteric cavity—entoderm cells always being interposed—it seems probable that these entoderm cells aid in nourishing the egg. After the oöcyte or young egg is formed and before it has begun to grow to any marked extent, it has the structure already described, viz., compact, granular, deeply staining cytoplasm with a large nucleus and nucleolus (figs. 7 and 8).

In the egg which has started its growth or in which a period of rapid growth is just initiated certain changes become noticeable; these are illustrated in figures 9 to 13, which represent a series of eggs of different stages of growth, all from the same general region of one colony. The eggs represented in figures 9 and 10 are from the stem of the hydroid close to a lateral branch which bears gonophores; they are just entering upon the period of rapid growth. The chromatin of their nuclei is in a more obvious network, the nucleolus more or less vacuolated, but sometimes homogeneous and without vacuoles (fig. 10), the cytoplasm not so dense as formerly, but still not alveolar, without yolk, but containing a scattered lot of prominent and intensely staining bodies. These bodies are of nuclear origin and are similar to the granules found in the cytoplasm of Clava, Campanularia, and Aglantha, which were derived from the chromatin of the nucleus.

While the egg is small these bodies are more or less scattered through the entire mass of the cytoplasm. When growth is well under way and proceeding rapidly, this extranuclear chromatin is massed close to the nucleus (fig. 11) and does not extend far into the cell body. This may indicate a rapid absorption of the material by the cytoplasm. The cytoplasm at such a period is more loosely arranged and shows a beginning of an

alveolar structure; the nucleus contains a more marked reticulum of chromatin, and the nucleolus is much vacuolated. Such an egg as that shown in figure 11 would be found in the pedicel or in the base of the hydranth which is producing gonophores. Figure 12 is an egg which has just reached the young developing gonophore, i.e., it is in almost its final position where it ripens and develops. The cytoplasm is noticeably alveolar, not deeply staining because of so loose a texture, and with a ring of chromatic granules close to the nucleus which is abundant enough to indicate considerable movement of nuclear substance into the cytoplasm. The nucleus does not differ from the earlier condition save for an increase in size and an increase in the amount of chromatin which results in a more marked reticulum.

The egg shown in figure 13 is located in the gonophore and is well started upon its growth, though not yet more than one-fifth of the final volume of a ripe egg. It differs from a mature egg mainly in size, the structure being quite similar. Large yolk spheres occupy most of the cytoplasm, the granular cytoplasm not being very abundant, while chromatin material is still passing from the nucleus in somewhat less abundance than formerly. The nuclear reticulum shows regions of concentration of chromatin at the nodes of the network, and a large vacuolated nucleolus similar to that of ealier stages is present, but not represented in figure 13. In later growth the changes from the above description are not great, yolk is produced to an enormous extent and the egg is loaded down with it. The nucleus probably does not change greatly, though it was not studied in the later growth phases. After the egg reaches the young gonophore the latter elongates, carrying the egg away from the hydranth and at the same time finger-like extensions of the stem surround the egg and supply nourishment to it during its later growth. The growth is completed, and maturation, fertilization, and development all take place within the gonophore, an embryo in the form of a planula finally escaping.

3. OBSERVATIONS UPON SPERM CELLS

Male gonophores are produced upon different colonies than the female gonophores and differ from them in appearance and somewhat in structure. So far as my observations go, I can confirm the conclusions of Weismann ('83) that the sperm cells of Eudendrium arise in the stem or stalk of the hydroid from entoderm cells and migrate into the gonophore. The growth of the gonophore also aids by passively carrying these cells into position.

A brief account of the formation of the gonophore is necessary in order clearly to understand the significance of germ-cell formation. The growing point of the hydroid is in the stem, immediately below the terminal hydranths where the cells are richly protoplasmic and the ectoderm and entoderm not differentiated into gland cells, nettling cells, and the like. The narrow, growing zone is followed proximally by the usual coenosarcal tissue with its differentiated cells of various sorts, both in ectoderm and entoderm. Newly formed lateral hydranths and gonophores develop at a point some distance proximal to this growing zone and their origin may be recognized, before there is any actual new growth, by the change in the character of the cells. This recognizable change is the production of a new growing zone in the coenosarc at that point, and this involves a regressive change of the differentiated ectoderm and entoderm into the same small, richly protoplasmic cells which are found in the growing point of the main stem. After this change has occurred the perisarc opposite this group of cells is dissolved, an evagination of the coenosarc occurs and gradually a new structure is differentiated from this bud.

Thus in the production of gonophores as well as of hydranths, differentiated cells undergo a regressive change and after further growth once more becomes differentiated. There is no difficulty in demonstrating this typical method of growth in female as well as in male colonies. This is very significant, when it is also found that the male germ cells arise in the stem adjacent to the gonophore, apparently from regular entoderm cells. If active, growing, undifferentiated cells may, by differentiation,

become typically functional ectoderm and entoderm cells, then later undergo a regressive change and become typical growing cells which results in the formation of a new polyp after a later second differentiation, there is nothing strange in thinking of similar regressive changes of entoderm resulting in the formation of germ cells.

The earliest indication of cells clearly distinguishable as sperm mother-cells was found in the entoderm of the stem of the hydroid not far distant from the base of the pedicel of a developing gonophore. Figure 14 shows these cells in the entoderm, with rather deeply staining cytoplasm and large nuclei which contained a good deal of chromatin concentrated close along the nuclear membrane. A large nucleolus was present. These sperm mother-cells arise in relatively small groups deep in the entoderm, but whether by modification of entoderm cells or by division of entoderm cells could not be determined. Such cells divide rapidly to produce larger masses of spermatogonia (fig. 15) which may be so numerous as to extend in unbroken lines from the stem along the pedicel and into the developing gonophore. As the growth of the gonophore continues, most of these spermatogonia are found in the gonophore itself and fewer in the pedicels and stems. Mitotic divisions are abundant in all such groups of spermatogonia, whether in the stem, pedicel, or developing gonophore, but most abundantly in the cells within the gonophore.

As the gonophore first forms, the spermatogonia are in continuous rows of groups, as shown in figure 17, but as growth continues and the gonophore becomes more definitely formed, these cells are more distinctly massed (fig. 18), and there is an ultimate localization into distinct testis-like groups (fig. 19). The character of the spermatogonial cells of young developing gonophores is shown in figure 16. Divisions are taking place, some growth is evident, but all are of the stage of spermatogonia. At about this period the cells undergo a synizesis-like change and rapidly thereafter transform into spermatozoa.

4. SUMMARY

In female colonies of Eudendrium egg cells appear to be widely distributed, but careful examination demonstrates their presence only in the smaller branches. They are always in groups located near the gonophores or in regions where gonophores would have formed in the living colonies. The egg cells originate in such positions, probably by modification of ectoderm cells, but they pass into the entoderm, where early growth takes place. The location of the eggs in the gonophores is due in part, probably, to an active migration and in part to a passive translocation caused by the growth of the tissues which go to form the gonophore. An escape of nuclear material, probably of chromatic nature, into the cytoplasm is coincident with the onset of the growth period in the egg. In the male colonies sperm cells arise in the deeper portion of the stems adjacent to, or in the region of later formation of, gonophores.

The germ cells of Eudendrium have commonly been discussed as arising very early in the history of the hydroid from a distinct germinal tissue, and much later, at the time of maturity, migrating into the reproductive bodies which are conceived as degenerate medusoid buds. Observations do not substantiate any of these interpretations, since the germ cells arise from ectoderm or entoderm cells just previous to the development of the gonophores, and the gonophores are not degenerate medusae, but, in female colonies at least, develop by a modification of ordinary polyps. There is no apparent phylogenetic significance in the manner, place, or time of formation of the germ cells of Eudendrium.

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ABBREVIATIONS

a, possible egg cells
ec., ectoderm
gl., gland cells
ov., egg cells
pr., perisarc of the hydroid stem
sp., male germ cells

PLATE 1

EXPLANATION OF FIGURES

1 Portion of female colony showing method of branching, position of hydranths and of gonophores. The dots in the stems represent the position of egg cells. *A*, *B*, *C*, are the portions of the stem shown in figs. 2, 3, 4, respectively. × 11.

2 Optical section of region *A*, fig. 1. The deeply staining cells in the entoderm include some egg cells and some gland cells. × 255.

3 Optical section from region *B*, fig. 1. The deeply staining cells in the entoderm are egg cells. × 255.

4 Optical section of stem from region *C*, fig. 1. Most of the shaded cells are egg cells. The presence of large and small eggs in the same region suggests their origin in this place; the shape of some eggs suggests a possible migration. × 255.

5 Optical section through a stem near the distal end of a colony, a region devoid of gonophores. It is difficult to say which of the deeply staining cells are gland cells and which eggs. × 473.

6 Optical section of stem and polyp, the latter becoming a gonophore. Egg cells present. × 150.

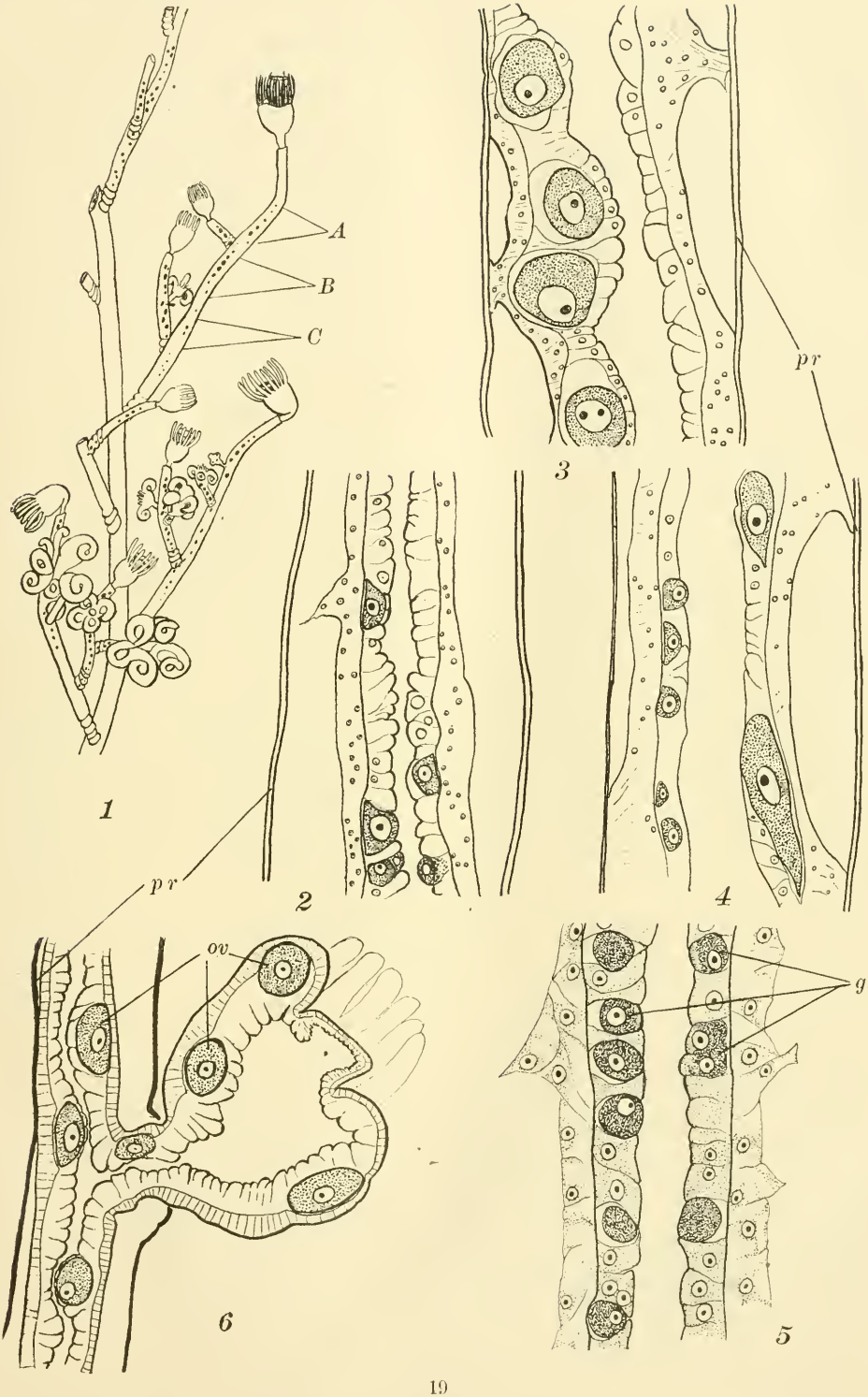

A
B
C
1
pr
2
3
pr
4
ov
6
gl
5

PLATE 2

EXPLANATION OF FIGURES

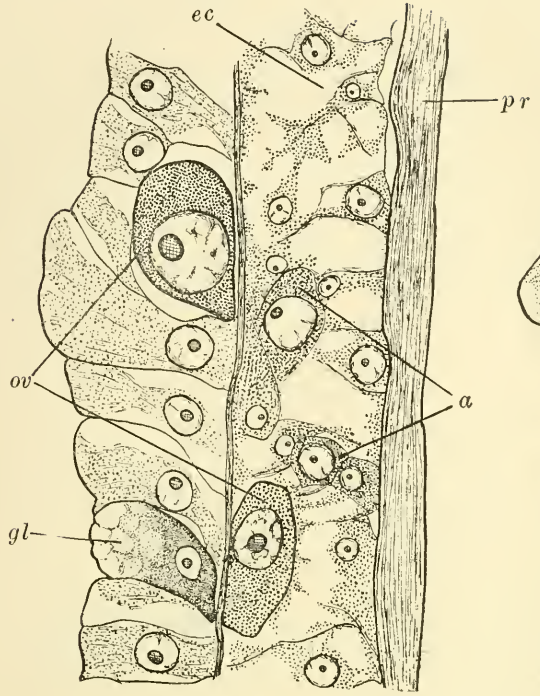
7 Longitudinal section of stem just below a terminal hydranth. Growing egg cells, *ov.*, are present both in ectoderm and in entoderm. The cells in the ectoderm marked *a* may possibly be primitive egg cells. × 1010.

8 Longitudinal section of the same stem as previous figure, showing two egg cells in the entoderm. Other similar cells are found both in ectoderm and in entoderm. × 1010.

9 Transverse section of stem at junction with the pedicel of a gonophore, showing a large growing egg in the entoderm and a cell, *ov.*, in the ectoderm which is suggestive of the early formation of a primordial germ cell. × 1010.

10 Growing egg cells from entoderm of stem. Nuclear material is passing into the cytoplasm. × 1765.

11 Growing egg from the same colony as previous figure. Egg located in the entoderm at the base of the hydranth which bears the gonophores. Prominent collection of nuclear material in the cytoplasm near the nucleus; the cytoplasm is becoming slightly vacuolated. × 1765.



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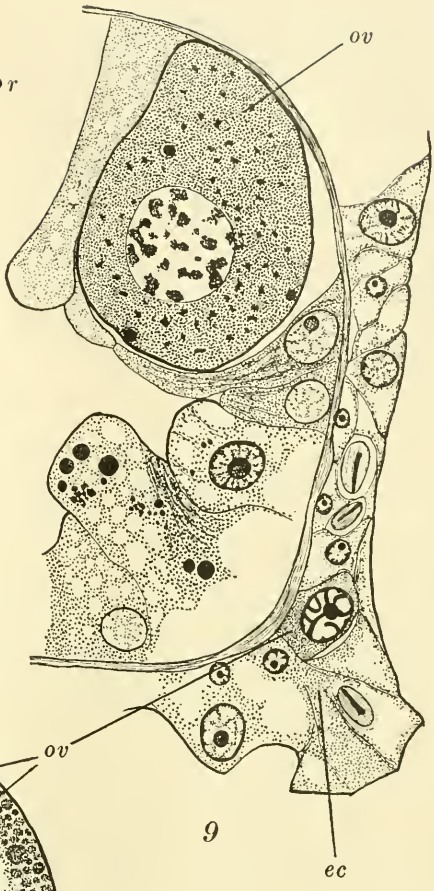

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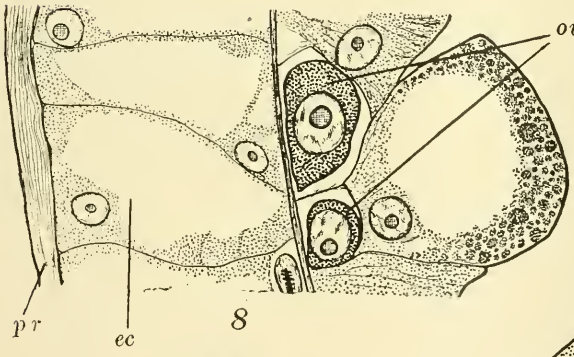

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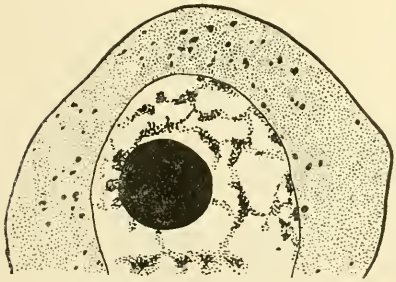

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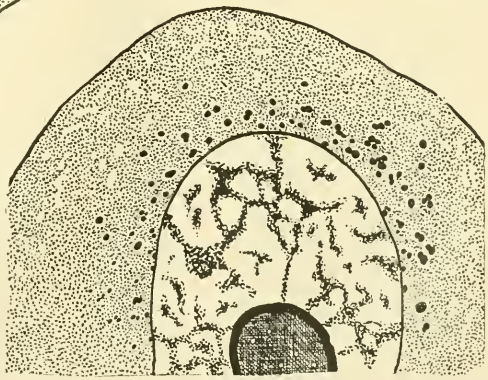

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PLATE 3

EXPLANATION OF FIGURES

12 Growing egg from a gonophore of the same colony as those of figures 10 and 11. Cytoplasm rather loose and somewhat alveolar with nuclear substance in the cytoplasm. × 1765.

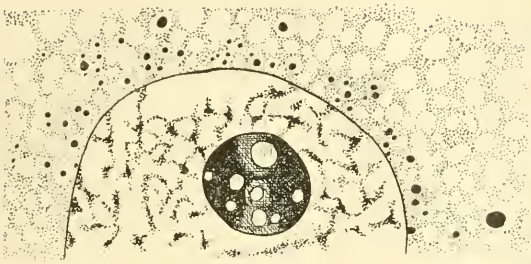
13 Larger egg from another gonophore of the same region as that of figure 12. The cytoplasm is filled with large yolk spheres, and the nuclear substance is still passing into the cytoplasm, though less abundantly than previously. A nucleolus is present in this egg, but was not shown in this section. × 1765.

14 Male colony. Longitudinal section of stem below the pedicel of a gonophore, with a small group of germ cells in the entoderm. × 1010.

15 Male. Longitudinal section of a stem just below the terminal hydranth and above a lateral branch which is forming gonophores. The entoderm contains a mass of cells, the deeper ones of which are primitive sperm cells. × 1010.

16 Longitudinal section through a developing male gonophore showing a group of spermatogonia in the entoderm. × 1010.

17 to 19 Longitudinal sections through developing male gonophores. The germ cells are shown in the pedicels and in the bodies, more or less scattered in figure 17, but becoming definitely localized in figure 19. × 255.



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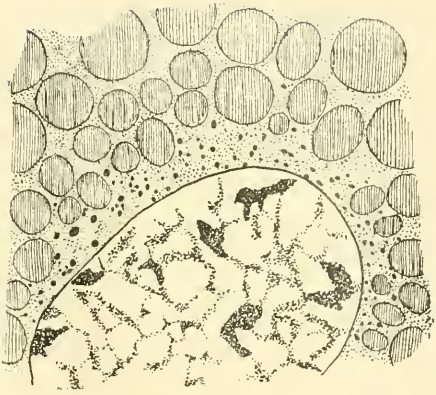

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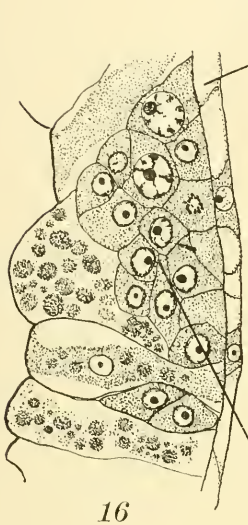

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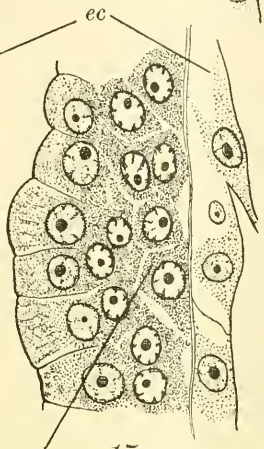

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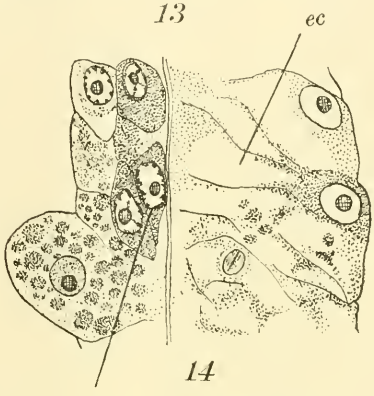

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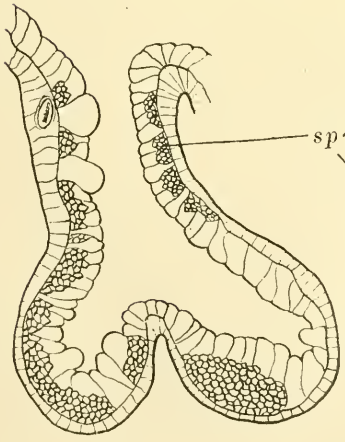

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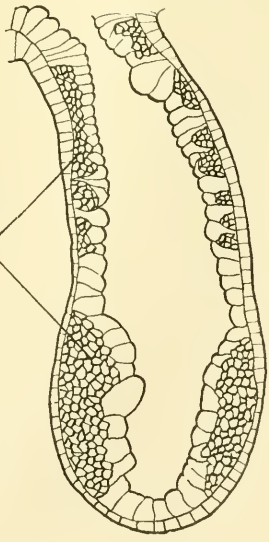

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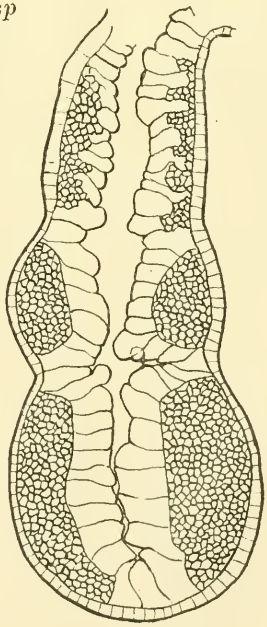

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE BRANCHIAL DERIVATIVES OF THE PIED-BILLED GREBE, WITH SPECIAL CONSIDERATION OF THE ORIGIN OF THE POSTBRANCHIAL BODY

CHARLES EUGENE JOHNSON

From the Department of Animal Biology of the University of Minnesota

THIRTEEN FIGURES

INTRODUCTION

The development of the thymus and associated derivatives of the gill pouches has been fully investigated in but a very few species of birds. Recently our knowledge has been extended by Helgesson's ('13) work on the common house sparrow, Passer domesticus, and by the study of Hamilton, of the same laboratory, on the development of the thymus in the duck. Preceding these among the more recent literature there is to be mentioned the work of Kallius ('05, '06), in which the gill pouches are treated in connection with the development of the tongue in the duck and in the house sparrow, and that of Rabl ('07) on the origin of the postbranchial body in the duck.

Some years ago a study of the development of the thymus[1] in the pied-billed grebe, Podilymbus podiceps (Linn.) was undertaken by me, with rather inadequate material. Recently, with additional and also more advanced stages, this study has been repeated, and since the grebe represents a low order of present-day birds and one not previously investigated in this connection, the later results, which in some respects differ from those of the first attempt, are here submitted as a slight addition to the subject of the gill pouches and their derivatives in birds.

[1] The development of the thymus in the pied-billed grebe. Subject of a thesis forming part of the requirements for the master's degree, under the supervision of Prof. H. F. Nachtrieb. Abstract appeared in Science, vol. 27, 1908.

REVIEW OF LITERATURE

The results obtained by different investigators of the development of the thymus in the chick vary. Van Bemmelen ('86) and DeMeuron ('86) came to the conclusion that the thymus here is derived from the third and fourth gill clefts. Kastschenko ('87) held that it is formed not only from the third and fourth gill pouches, but also from the fifth, while Mall ('87) asserted that the gland is a product of the third visceral pouch only. More recently the subject has been thoroughly investigated by Verdun ('98) who found the thymus in the chick to arise from the third and fourth visceral pouches, the derivative of the latter pouch being somewhat rudimentary. Keibel and Abraham (quoted from Helgesson) in their Normentafel, 1900, also show the thymus of the chick as a derivative of the third and fourth pouches.

In the duck and the sparrow, according to the studies of Hamilton and Helgesson respectively, the thymus is formed from the third visceral pouch only.

Parathyreoid bodies occur in all species of birds investigated. According to Van Bemmelen, De Meuron, and Mall, in the chick, the third and the fourth visceral pouches each gives rise to a parathyreoid body, and the same has been found true by Hamilton and Helgesson for the duck and the sparrow. Verdun, in the chick, found a third parathyreoid body arising in connection with the postbranchial body; this was of rather rudimentary nature and enclosed within the postbranchial organ. It has been interpreted as representing a parathyreoid derivative of the fifth visceral pouch.

The postbranchial bodies arise as paired structures behind the fourth visceral pouches, and, with the exception of Rabl ('07), whose work will be considered later, are generally held to be derivatives of the fifth visceral pouches in birds. In the chick, according to Mall, the postbranchial bodies, which at first form epithelial vesicles, gradually break down after the tenth day, and at the end of the period of incubation, form the mass of granules which "Remak saw surrounding the fourth aortic arch." Verdun, however, states that at about the tenth day these bodies

assume the characteristic structure of postbranchial bodies, consisting essentially of compact epithelial cords and lobules, and a varying number of globular vesicles with walls of cubical or cylindrical epithelium. In the duck Verdun and Hamilton describe conditions virtually the same as in the chick. In the sparrow, on the other hand, Helgesson found that the postbranchial body of the right side atrophies, having entirely disappeared in embryos of 14 mm. length. That of the left side persists, and was observed in full-grown individuals.

THE BRANCHIAL DERIVATIVES IN THE GREBE

In grebe embryos of about six days the third gill cleft has been shut off from the exterior by the closing of the ectobranchial duct III (terminology of Hammar, '13); its pharyngeal passage or the entobranchial duct III may be entirely or only partly occluded. The fourth visceral pouch extends to the ectoderm, with which its lateral wall is in intimate contact. The entobranchial duct IV, which is still open, enters the pharynx, caudal to and entirely independently of the entobranchial duct III, and that of the left side bears laterally a well-developed spherical vesicle, the postbranchial body. On the right side this body is less clearly differentiated. Both third and fourth gill pouches are situated somewhat obliquely and are flattened under the influence of the aortic arches so that they present posterodorsal and antero-ventral surfaces.

At six and one-half days the changes in these two pouches consist chiefly in their having become somewhat longer, with further reduction of the ectobranchial and entobranchial ducts. Both postbranchial bodies are well developed and have become somewhat fusiform, with thick walls and a narrow, but sharply defined central canal. The right postbranchial body at this stage reaches its maximum development.

In embryos of about six and three-quarter days marked changes are noticeable. The third visceral pouch has completely separated from the pharynx and forms a V-shaped body whose apex is directed laterally and is attached to the ectoderm by the short solid ectobranchial duct. From the apex one limb of the

V extends anteromedially across the ventral surfaces of the jugular vein and vagus. This limb is a solid cylindrical mass and is the anlage of the thymus III. It represents a rapidly proliferating area, including the anterior and dorsolateral walls of the pouch. A continuation of the thymus anlage forms the apex of the V, and the ectobranchial duct is connected chiefly, if not wholly, with this part of the pouch, but the exact limits of ectoderm and entoderm cannot be definitely made out. The other limb of the pouch is a somewhat flask-shaped vesicle with walls of uniform thickness in the enlarged part; the neck portion is the drawn-out pharyngeal end of the pouch, which is hollow throughout and extends caudo-medially across the space between the third and fourth aortic arches. Another embryo of the same age shows the next step in the development of this pouch. It has here separated from the ectoderm and straightened out, lying lengthwise in the neck, just ventral to the jugular and the vagus and lateral to the third aortic arch. The thymus portion can now be followed as a ridge-like thickening, nearly to the posterior extremity of the pouch. The vesicular portion is somewhat flattened laterally and its narrow dorsal and ventral walls consist as yet of unthickened epithelium; the medial wall, on the other hand, has become distinctly thickened and constitutes the first trace of the parathyreoid body III.

The fourth visceral pouches at six and three-quarter days are completely detached from the ectoderm, and that of the right side has also separated from the pharyngeal wall. These pouches are scarcely half the size of the third, and have a rather short, club-shaped form, the anterior ends tapering more or less and pointing toward their former ectodermal connections. The pouches have a relatively large central cavity and the anterior wall is somewhat thickened, but otherwise no regional differentiations are noticeable.

On the left side a rather misleading situation has arisen in the relations of the third pouch and the postbranchial body (figs. 1 and 2). The latter is still attached to the pharyngeal wall by a slender remnant of the entobranchial duct, but lies further cephalad than in the preceding stages, being now opposite the

interspace of the third and fourth aortic arches instead of the fourth and fifth. Its posterior terminal portion is in such close apposition to the medially directed end of the third pouch, that only by careful examination under high power of the microscope can it be determined that the two structures are not actually continuous. The relations of the postbranchial body to the third pouch are thus in this stage almost identical with the earlier relations of the body to the fourth pouch, and create an appearance of genetic connection with this pouch—an appearance that becomes all the more convincing in the absence of proper intermediate stages. The forward movement of the postbranchial body is evidently the result of traction exerted by the growing pharyngeal tube. The right postbranchial body has been reduced to a slender strand of loosely associated cells, in some parts of which, however, traces of the original lumen may still be discerned.

At seven days the process of separation between the thymus and parathyreoid III has made considerable advance. The entire pouch may, in some cases, have rotated to a certain degree on its long axis, bringing the parathyreoid into a more nearly ventral position. The thymus III (fig. 11) is an elongate compact body, thicker and nearly cylindrical in its anterior portion, and flatter posteriorly where it is united to the parathyreoid by its medial or ventral surface. The parathyreoid contains a small lumen whose immediate walls may in some cases consist of a single layer of low cells, overgrown by adjacent rapidly proliferating areas; in others the proliferating process has extended so that the lumen appears merely as a slit in the mass.

The fourth pouch presents a form very similar to the third, but is much smaller, being but slightly longer than the parathyreoid portion alone of the latter pouch; this great difference in relative size has resulted from the rapid growth of the thymus portion of the third. The entire pouch is shown in medial surface view in figure 12, from a model made to the same scale as that illustrated by figure 11. To be noted is the division of the pouch into two main masses which have the same relation to each other as the thymus and parathyreoid of the third pouch.

The lateral mass, which is solid, is the thymus of the fourth pouch (thymus IV); the medial body, consisting of cord-like cellular masses and containing a small cavity near its postero-lateral surface, is the parathyreoid IV. Figure 3 is a transverse section through the fourth pouch of another embryo of seven days, taken slightly anterior to the middle of the pouch, where the thymus and parathyreoid are well differentiated from each other and are being separated. The structural difference is apparent. The thymus portion in this specimen is slender and cylindrical and somewhat smaller relatively to the parathyreoid than that of the preceding embryo, but, like the latter, can be traced as a ridge-like thickening in the dorso-lateral wall of the pouch, throughout its length, presenting a duplication on a smaller scale of the conditions existing in the third pouch. The close analogy will be seen by comparing figure 4, which is a section through the posterior, vesicular portion of the fourth pouch, with figure 5, a section through the corresponding region of the third pouch in another embryo. The degree of differentiation in the two pouches is nearly the same.

The postbranchial body has lost its connection with the pharynx and is now a closed vesicle, of fusiform shape, with clear-cut lumen. Its position is still medial to the interspace of the third and fourth aortic arches, at the level of the oesophagus.

In embryos of eight and one-half days the thymus III is a narrow, greatly elongated body whose caudal third, approximately, lies in the original position of the gland, just ventrad of the jugular vein, while the anterior, greater portion, which is more or less irregularly lobed, pursues a spiral course about the lateral side of the vein and comes to lie along its dorsolateral surface; the caudal portion is very slender and its extremity is narrowly attached to the parathyroid III. The final separation of the two bodies is apparently accomplished soon after the ninth day, in the manner suggested by Helgesson for the sparrow, by a process of atrophy and attenuation of the connecting portion of the thymus.

The thymus IV has correspondingly increased in length and lies along the lateral side of the thymus III (fig. 6). At some

points the two bodies are in close contact and are in the process of fusing, at others a distinct space separates them. The size of the thymus IV at this stage, though somewhat variable, is hardly more than a fourth that of the thymus III. In one embryo the thymus IV has separated from its associated parathyreoid, in others the two are still narrowly connected, similarly to the derivatives of the third pouch. At this stage the conditions in the grebe, with respect to the derivatives of both third and fourth pouches, correspond closely to those shown by Verdun for his chick embryo of one hundred and eighty-eight hours.

In embryos of nine days the thymus derivatives of the third and fourth pouches are already fused. The double nature of the resulting organ cannot in some cases be detected from its appearance in the sections, but in others the two parts are as yet easily discerned. The complete amalgamation of these derivatives is a relatively rapid process and the thymus anlage of the fourth pouch may, in the absence of the proper developmental stages, be readily interpreted as of merely transitory nature.

The parathyreoids III and IV at this stage lie side by side upon the dorsolateral surface of the thyroid. The parathyreoid III is medial in position and is considerably larger. The two are so closely apposed that the division between them can be made out only under high-power examination. In each of the bodies of the right side traces of the original cavity still are found. In the parathyreoid III the lumen is small, extending through 60 micra, but is sharply defined. It is situated near the lateral edge of the body and is enclosed by a single layer of unthickened epithelium, forming a vesicle partly surrounded by the modified, cord-like masses of the gland. On the left side there are no certain indications of the original cavities or of unmodified epithelium in either of the parathyreoids.

The postbranchial body in embryos of seven and one-half or eight days may be partly or completely broken down into a mass of loosely arranged cells, which is but poorly differentiated from the surrounding mesenchyme. Because of this fact I came to the conclusion in my earlier study that the body disappeared soon after this period. Later stages at present available show

that such is not the case. In embryos of nine days the cellular mass has grown very considerably larger and has become more compact structurally. It is very irregular in form and its position is changed so that it now lies just dorsal to the thyreoid-parathyreoid group, but it extends somewhat beyond this group posteriorly and encircles the fourth aortic arch from the medial, posterior, and lateral sides; a portion of it also has grown in between the thyreoid and parathyreoids. At twelve days the disposition of the mass is in general much the same as at the ninth day and there is no marked increase in extent, but the mass as a whole is more clearly delimited. The greater part of it lies in close contact with the dorsal surface of the thyreoid, with no intervening connective tissue layer. There are no ramifications entering the thyreoid gland, however, and the same is true for the parathyreoids, but these two are now enclosed in a common connective tissue sheath and are not in direct contact with the postbranchial mass. The posterior portion surrounds the brachiocephalic trunk near the origin of the subclavian artery. The gland contains as yet no indications of epithelial vesicles.

Because of its place relations to the organs under consideration, the carotid gland is to be mentioned. This gland becomes clearly recognizable in grebe embryos of about eight and a half days. It then lies at the dorsolateral side of the carotid artery, just cephalad of its junction with the subclavian. Its posterior portion is in close proximity to the arterial wall, but anteriorly the gland extends obliquely laterad, away from the vessel, and lies near the dorsolateral surface of the thyreoid, with the parathyreoid group bounding it laterally (fig. 9). On the left side, by the presence of the postbranchial body medially and dorsally, the gland becomes quite surrounded.

THE ORIGIN OF THE POSTBRANCHIAL BODY

In an investigation of the early stages of the gill pouches in the duck, Rabl ('07) has described what he recognizes as a sixth visceral pouch, represented by a diverticulum appearing in embryos of about four and a half to five days, on the caudo-medial wall of the pharyngeal evagination which is generally held to be

the fifth visceral pouch in birds. The diverticulum in question later gives rise to the postbranchial body, which, therefore, in Rabl's view, is not a derivative of the fifth pouch.

Rabl's work seems to have been overlooked by Hamilton in his study of the derivatives of the gill pouches in the duck, and his attention apparently was not directed to a closer study of the relationship of the postbranchial body to a possible sixth, as well as to the so-called fifth visceral pouch. He does not allude to similar observations in connection with his specimens of three and a half, five and a half, and six days, and he derives the postbranchial body from what he refers to as the medial of two globular parts into which the fourth visceral pouch has become divided by a constriction about its middle portion.

In grebe embryos of four and a half, five and five and a half days I find conditions very similar to those observed by Rabl in duck embryos of approximately corresponding ages. The relations of the parts concerned are most clearly shown in the five-day stage (fig. 13). On the caudo-medial wall of the fourth visceral pouch are two well-defined diverticula, one medial, the other lateral. The medial diverticulum is larger and deeper and is situated at the junction of the fourth pouch with the pharyngeal wall. The area which the two diverticula together occupy is clearly marked off from the fourth pouch itself, more so on the left side, where, as previously noted, the postbranchial body attains greater development than on the right. In the light of later stages, the medial of the two diverticula gives rise to the postbranchial body and corresponds to the sixth pouch of Rabl; the lateral one represents the fifth. The relations of these two pouches to each other, to the fourth pouch, and to the pharynx are essentially the same as those described by Rabl for duck embryos of five days and sixteen hours and five days and eight hours, represented by his text figures 5 and 6, respectively. (That we are dealing with corresponding parts in the two species will become further evident by referring to Rabl's work and comparing his figures 4 e, 4 f, Pl. XI with figures 7 and 8 of the grebe.) The mesenchymal ridge (*V.a.VI*) between the fifth and sixth pouches is the sixth visceral arch of Rabl, and lateral to

it, between the fourth and fifth pouches is the fifth visceral arch (*V.a.V*). Kallius ('05) had previously noted the sixth mesenchymal ridge and had observed a small blood-vessel in it, but was not prepared to state whether or not this vessel represented an aortic arch. Rabl, however, found the rudimentary sixth aortic arch in close association with this ridge, sometimes in it, sometimes at one side, and his conclusion that the ridge is the sixth visceral arch rests in part upon this fact.

The youngest embryo in my series is one of forty-six hours. At this stage a division of the so-called fifth pouch is not distinguishable, although this diverticulum is prominently developed and opens more directly into the pharyngeal cavity than is the case in Rabl's duck embryo of four days and seven hours, in which the fifth and fourth pouches are at the end of a common outpocketing of the pharyngeal wall. In the forty-six-hour grebe the diverticulum of the fourth pouch extends ventrally beyond the fifth pouch as a blind pocket. The first four aortic arches are present in full length from truncus arteriosus to aortic root (fig. 10). The fourth is the smallest, and the left one is considerable smaller than the right. Shortly after leaving the aortic root the fourth arch on each side gives off a branch which courses along the posterior wall of the fifth visceral pouch and can be traced ventrally about half the distance to the truncus arteriosus. Near its origin each of these vessels in turn has a small, short branch directed so that, if extended, the vessel would pass ventrally along the border line between the fourth and fifth diverticula. From the position of these vessels it is evident that the posterior larger one is the sixth aortic arch and the anterior imperfectly developed branch is the fifth. So far as the evidence from this stage goes, it points to a condition of these vessels entirely in accord with that in the duck. The available stages however, are not adequate for ascertaining the relation of the sixth aortic arch to the corresponding pouch and visceral arch during all of the subsequent development of these structures.

An embryo of four and a half days presents what appears on casual examination to be an undivided diverticulum corresponding to the fifth pouch of the forty-six-hour stage. A more careful

scrutiny of the sections, however, reveals on one side a shallow but distinct groove on the outer surface of the diverticulum, and on the opposite inner surface is a low ridge. This feature can be traced through approximately the caudal half of the total number of sections through which the diverticulum extends. On the opposite side, although not apparent in the sections, a tendency to division of the pouch is shown in a reconstruction made to a magnification of 100 diameters. The lines of division here indicated correspond to those separating the fifth and sixth pouches in the five-day embryo and evidently denote the beginning of this differentiation. The time of appearance of this division well accords with Rabl's observations on the duck, where, though not present in an embryo of four days and seven hours, it was conspicuous in one ten hours older.

The duration of the fifth visceral pouch is rather brief. In an embryo of five and a half days (fig. 7) it already is more flattened and is much less distinctly differentiated from the fourth pouch, but it is still sharply marked off from the postbranchial body which has increased in size and protrudes conspicuously. By the sixth day the fifth pouch has been further reduced, so that it is recognizable merely as a slight elevation in the dorsal wall of the fourth visceral pouch, just lateral to the postbranchial body. From the last named it is still distinctly demarcated, and it clearly does not take part in the formation of this body. The evidence from these stages indicates, therefore, that the fifth pouch becomes separated from the postbranchial body along with the fourth visceral pouch. Beyond this period I have not been able to distinguish it and it is probable that, as suggested by Rabl, it eventually becomes a part of the connecting stalk which for a time unites the fourth pouch and the postbranchial body.

The degree to which the fifth and sixth pouches may become differentiated from each other in birds is possibly variable for different groups. Where the fifth visceral arch is poorly developed the corresponding gill pouch must have more nearly the appearance of a secondary lobe of the fourth, and the distinction between the fifth and sixth, in turn, may then become more or

less obscured. Since even where these last two become clearly differentiated as separate diverticula, the period during which they remain so is comparatively brief, this phase in their development may readily escape observation.

SUMMARY

The thymus in the pied-billed grebe is derived from the third and fourth gill pouches. In each pouch the anlage arises from a proliferating area beginning in the anterior wall and continuing in the dorsolateral wall to the posterior end of the pouch. The larger part of the thymus is contributed by the third pouch. The much smaller derivative of the fourth pouch becomes fused with the caudal portion of the thymus III at about the ninth day, and at twelve days the mass resulting from the fusion has greatly increased in size and its connection with the remaining part of the thymus has been reduced to a narrow band.

A parathyreoid body is formed from each of the third and fourth gill pouches. Their anlagen, which appear soon after those of the thymus, arise as thickenings of the medial walls of the pouches, but eventually all of each pouch, except the thymus portion, becomes converted into the parathyreoid body. Soon after the ninth day the parathyreoids become separated from the thymus bodies and assume a position upon the dorsolateral side of the thyreoid, the larger parathyreoid III being medial, the smaller parathyreoid IV, lateral. At twelve days they still have the same position, and are closely apposed and enclosed in a common sheath of connective tissue.

Of the postbranchial bodies the left alone persists, the right having disappeared by about the seventh day. At twelve days the persisting body is a relatively large irregular mass lying upon the dorsal side of the thyreoid, its posterior portion encircling the brachiocephalic trunk. Epithelial vesicles are as yet not formed.

The postbranchial body arises from the median of two diverticula into which the so-called fifth visceral pouch of birds becomes divided, and which Rabl in the duck has interpreted as a sixth

visceral pouch. The lateral of the two diverticula, or the fifth pouch, becomes entirely incorporated in the fourth pouch or its connecting stalk. No evidence was found that it participates in the formation of the postbranchial body.

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PLATE 1

EXPLANATION OF FIGURES

1 Transverse section through the postbranchial body and the posterior portion of the third visceral pouch of a grebe embryo of six and three-quarter days. × 50.

2 A section from the same series, through the posterior end of the postbranchial body, showing its close association with the pharyngeal end of the third pouch. × 210.

3 Transverse section of the thymus and parathyreoid derivatives of the fourth visceral pouch of a grebe embryo of seven days. × 210.

4 A section from the same series, taken near the caudal end of the pouch. × 210.

5 Transverse section near the caudal end of the third pouch of a grebe embryo of seven days. × 210.

6 A nearly horizontal section through the thymus derivatives of the third and fourth pouches of a grebe embryo of eight and one-half days. × 50.

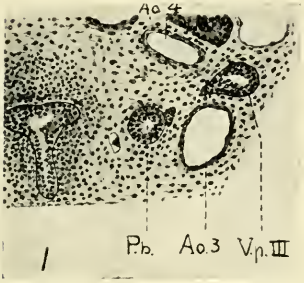
7 Transverse section of a grebe embryo of five days, showing the fourth, fifth, and sixth pouches of the left side. × 50.

8 Transverse section of a grebe embryo of five and one-half days, showing the same pouches as figure 8, the section cutting the posterior wall of the sixth pouch. × 50.

9 Transverse section of a grebe embryo of twelve days, showing the position of the postbranchial body with respect to the thyreoid, parathyreoid, and carotid glands. The section is taken just behind the parathyreoid IV, which therefore is not shown. × 30.

ABBREVIATIONS

Ao.3,4,5,6, third, fourth, fifth, sixth aortic arches
C.gl., carotid gland
Car., carotid artery
Par.III,IV, parathyreoid of the third and the fourth visceral pouch.
P.b., postbranchial body
Phar., pharynx
Thy.III,IV, thymus of the third and the fourth visceral pouch
Thr., thyreoid
V.a.V,VI, fifth and sixth visceral arches
Vag., vagus nerve
V.p.III,IV,V,VI, third, fourth, fifth, sixth visceral pouches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P.b.
Ao.3
V.p.III
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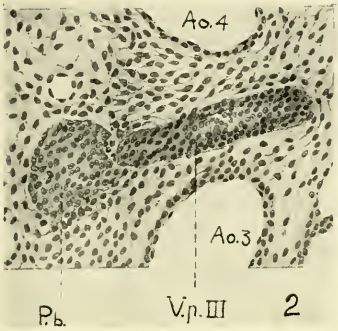
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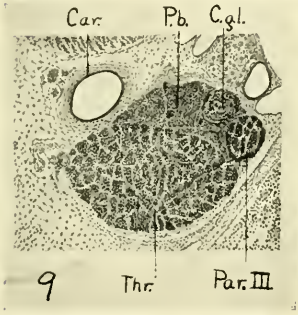
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P.b.
C.g.l.
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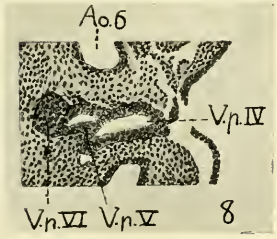
Thr.
Par.III


Ao.6
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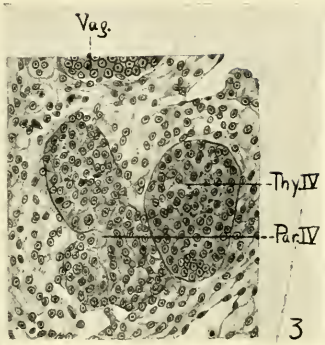
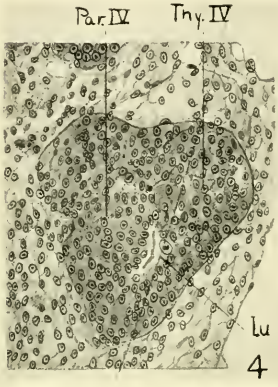
V.p.VI
V.p.V
8


Vag.
Thy.IV
Par.IV
3

Thy.III
Thy.IV
Thr.
6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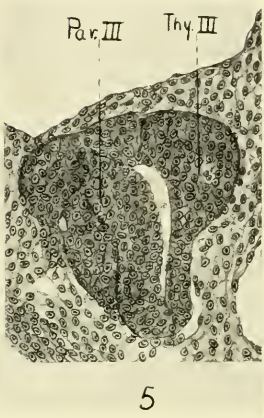
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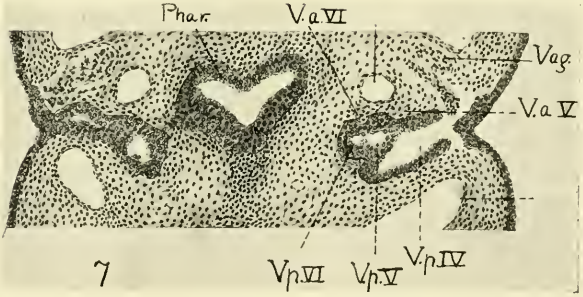

Phar.
V.a.VI
Vag.
V.a.V
7
V.p.VI
V.p.V
V.p.IV

PLATE 2

EXPLANATION OF FIGURES

10 Horizontal section through the gill pouches of a grebe embryo of forty-six hours, showing the position of the developing fifth and sixth aortic arches. × 66.

11 Medial view of a wax reconstruction of the third visceral pouch of a grebe embryo of seven days. × 133⅓.

12 Medial view of a wax reconstruction of the fourth visceral pouch of the same embryo. × 133⅓.

13 Postero-ventral view of a wax reconstruction, showing the fifth and sixth visceral pouches in their relations to the fourth pouch and the pharynx, in a grebe embryo of five days. × 133⅓.

ABBREVIATIONS

Ao.3,4,5,6, third, fourth, fifth, sixth aortic arches

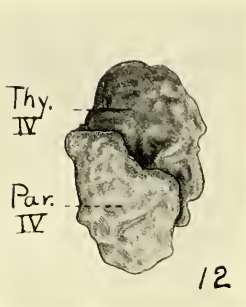
Par.III,IV, parathyreoid of the third and the fourth visceral pouch

Thy.III,IV, thymus of the third and the fourth visceral pouch

V.p.III,IV,V,VI, third, fourth, fifth, sixth visceral pouches

Drawings by G. H. Childs

Ao. 3
V.p.III
Ao. 4
V.p.IV
Ao. 5
Ao. 6
10


Thy.
IV
Par.
IV
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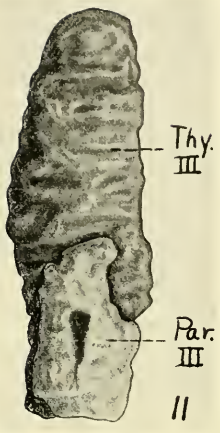
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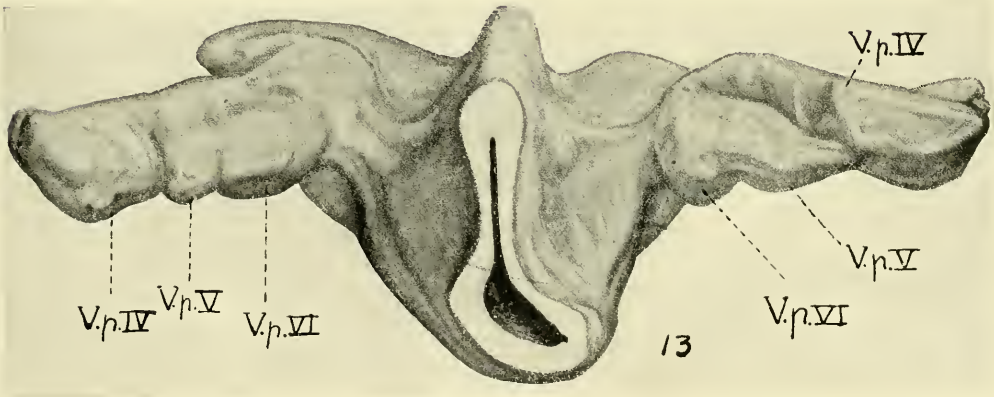
V.p.IV
V.p.V
V.p.VI
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V.p.VI
13

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LONGITUDINAL FISSION IN ACTINIA BERMUDENSIS VERRILL[1]

A. C. WALTON

Bermuda Biological Station for Research

EIGHT FIGURES

Longitudinal fission has long been recognized as a normal means of non-sexual reproduction among sea anemones. McCrady ('58)[2] observed aboral-oral fission in Actinia cavernosa, and G. C. Davenport ('03) observed similar phenomena in Sagartia luciae. In these cases fission was by constriction, followed by rupture. Carlgren ('93) in Protanthea simplex, and Torrey ('98) in Metridium found an aboral-oral fission by constriction without rupture. Mrs. Thynne ('59) observed a side-to-side fission accompanied by rupture in the case of Cyanthina smithi. Torrey and Mery ('04) show that all three of the above methods are normally found in Sagartia davisi, with the plane of division running perpendicular to the long axis of the mouth.

It is especially in Metridium that the double condition has been observed. Two-headed specimens had been noted as early as 1775 (Dicquemare). Johnston ('47) stated his belief that these conditions were due to the coalescence of two separate individuals; but Gosse ('60) contended that such individuals owed their existence to spontaneous division. Foot ('63) recorded the discovery of a specimen having two mouths on one oral disc, but expressed no opinion as to the cause. Carlgren ('93), like Gosse, contended that such forms were stages in a

[1] Contributions from the Bermuda Biological Station for Research, No. 87.

[2] For further literature see the following papers of Parker and Carlgren.

Parker, G. H., 1899. Longitudinal fission in Metridium marginatum Milne-Edwards. Bull. Mus. Comp. Zoöl., vol. 25, pp. 43–55, 3 pls.

Carlgren, O., 1909. Studien über Regenerations- und Regulationserscheinungen. II. Ergänzende Untersuchungen an Actinien. Kongl. Svenska Vetenskaps Akad. Handlingar, Bd. 43, No. 9, 48 pp., 4 Taf.

process of longitudinal division, and Torrey ('98) actually followed the changes of certain steps of this process in Metridium.

In working upon Metridium marginatum, Parker ('99) definitely showed that there is natural normal longitudinal fission, and that the double mouthed and double headed animals were but stages in such a division. Cases where there was circumstantial evidence of completed division were also found. The division plane was always through primary ectocoels or primary entocoels. Carlgren ('04) believed that, though *aboral-oral* fission could take place as the result of injury, *oral-aboral* fission did not occur, and that the double forms of Metridium studied by Parker and by Torrey were not stages of longitudinal division, but monstrosities developed from partially double embryos, such as are often found in Sagartia and Cribrina species. His further work (Carlgren, '09) only strengthened his belief that fission was due to regeneration after laceration, followed by division, and was not a normal mode of reproduction.

The material upon which the present article is based consisted of specimens of Actinia bermudensis Verrill obtained at Bermuda during the summer of 1916. I wish here to thank Dr. E. L. Mark, Director, and Dr. W. J. Crozier, Resident Naturalist, for the opportunity to study at the Bermuda Biological Station, and also for their personal assistance.

Actinia bermudensis Verrill is a small, blood red, shade-loving anemone, living between tide levels, and found only in certain caves along the shores of Bermuda. There are two color phases, perhaps varying enough to be classed as subspecies: one a bright blood red, the other slightly darker and more nearly prune-colored. That the darker colored forms are not merely old individuals is shown by the fact that the embryos of each type are colored exactly like their parents. Mature specimens of either type are from 3 to 4 cm. across the base, and from 2.5 to 3.5 cm. across the oral disc, while the column is 4 to 5 cm. in height. The animals are commonly hexamerous, having two pairs of directives, one pair to each siphonoglyph, and from ninety-six to one hundred and twenty mesenteries arranged in three orders. The column wall and both oral and pedal discs

are sufficiently transparent to show the attachments of the mesenteries. The inner edges of the complete mesenteries (below the level of the stomenteron) as well as those of the incomplete mesenteries are provided with mesenteric filaments. These at their bases give rise to long, much convoluted acontia. These organs are protruded through the mouth, and not through cinclides. Gonads are present on all of the mesenteries of the first and second orders, except the two pairs of directives; and a few traces of immature gonads were also found on some of the mesenteries of the third order. Only one ostium, the median, is generally present in the mesenteries of Actinia bermudensis.

The tentacles, arranged in two very irregular rows, are from eighty-five to one hundred and twenty in number in mature specimens, and at the bases of the outer row are twenty-four irregularly spaced, bright bluish-violet eminences, the acrorhagi.

This species of sea anemone was first described by Verrill in 1898, and since that time has been observed and worked upon by several investigators. No mention, however, has been made of specimens of a double nature, and, except for a single specimen kept in the laboratory for some time as a curiosity by Dr. Crozier, I believe the specimens obtained during the summer of 1916 are the first individuals having either double heads or double mouths that have been carefully examined. The specimen Dr. Crozier found had a double mouth and more than the ordinary number of tentacles, but it underwent no visible changes while under observation.

While working on the early embryology of Actinia bermudensis, I had occasion to examine carefully over a thousand specimens of all ages, and all but a very few were of the bright red variety. Among this number of animals were two which first attracted attention by their size and shape. The oral discs were greatly elongated, the long axis being almost twice the length of the shorter, which had normal dimensions. On examination it was found that one specimen (fig. 1) had two separate diglyphic mouths, the long axes of which made a slight angle with each other. In the other specimen (fig. 2) the mouth was in the process of division. The mouth opening had roughly the shape of

an arrow head, with three siphonoglyphs, one corresponding to the point of the arrow head, the other two to its barbs, the latter having arisen by a division of one of the original siphonoglyphs.

These two specimens, living in the laboratory in a tank of running sea-water, and fed on bits of crushed crab, were kept under observation for almost five weeks. Their development is of especial interest, inasmuch as they showed a slow, but evident, progressive fission longitudinally, which in one case ended in a sudden completion of the process by the 'constriction-and-rupture' method shown by Torrey and Mery ('04) for Metri-

Fig. 1 Photograph of Actinia *A*, oral view, showing two separate mouths.

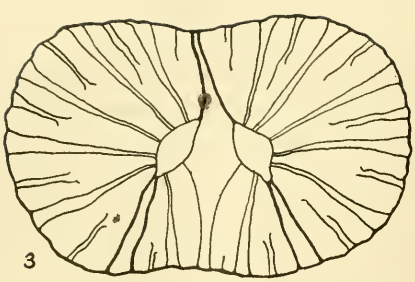
dium. In Metridium, however, no direct evidence of the fission of the double forms has as yet been recorded.

When specimen *A* (fig. 1) was fully expanded, the long axis of its oral disc measured 6.5 cm., and its pedal disc 7.5 cm. As in the case of Metridium, the number of tentacles was greater than normal. The normal number for an adult individual is about one hundred and ten, generally closely corresponding to the number of inter-mesenteric spaces. In this animal, when first found, there were one hundred and eighty-seven tentacles, and two more were formed before fission was completed. The transparency of the oral disc allowed the counting of the mesenteries

of the first and second orders with great accuracy. At the ends of the siphonoglyphs (fig. 3) two directives could be made out for each member of the most widely separated pair, while only one directive extended from each of the members of the other pair. A diamond-shaped area lying between the two mouths was entirely destitute of mesenteries. In all, thirty-two complete mesenteries were made out, of which twenty-eight were arranged in pairs, while four—two directives and two non-directives—were unpaired. Slight evidences of longitudinal infolding, especially at the oral end of the column, were apparent.

Three weeks later the infolding (fig. 4) was very evident, and the halves of the oral disc had almost completely separated. In place of each of the two unpaired directives there was now a paired directive, also in place of the two unpaired non-directives there were paired non-directives. One of the halves of the animal (right) had formed another pair of complete mesenteries. The opposite half had formed only a single complete non-directive, its mate being incomplete. During the next night rapid fission had begun, and by morning had proceeded about 1 cm. down the column. The fission continued rapidly and was complete eight hours later, the whole of the final separation taking from twelve to fourteen hours. The opposite walls of the column had infolded until only a narrow strip of material joined the halves. This narrow strip was ruptured by the straining apart of the halves, the split moving in an aboral direction. The ruptured surfaces were immediately rolled inward, and the animals contracted entirely, remaining in this state for five days. Upon opening again, the wounds were seen to be completely closed by a strip of salmon-pink tissue, 3 to 5 mm. wide, and running the whole length of the column. The two animals were then killed and dissected.

Figure 5 shows cross-sections of both animals through the region of the stomenteron. Daughter animal *x* (left in the figure) had both pairs of directives developed; the whole left half, which was that of the original specimen, was entirely normal, having five pairs of complete and eighteen pairs of incomplete mesenteries. The right half had formed three pairs, and one



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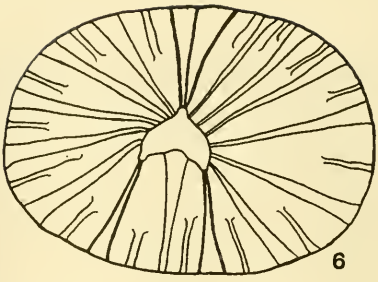

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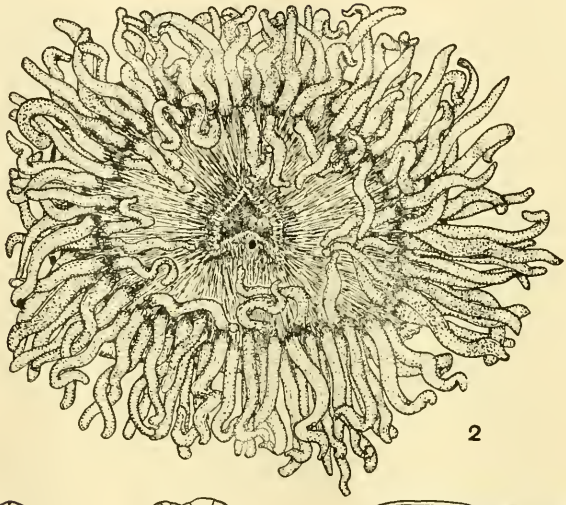

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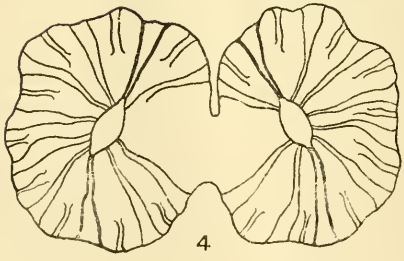

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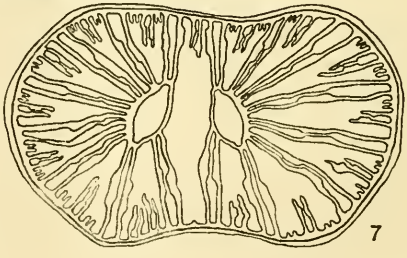

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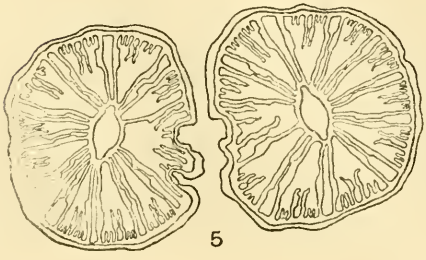

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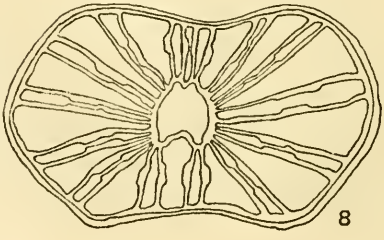

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member of a fourth pair, of non-directives. The fundaments of the other member of the fourth and of both members of the fifth pair of complete mesenteries had formed, as well as those of two pairs of incomplete mesenteries. The fundaments of the three complete and four incomplete mesenteries arose from the new tissue that had regenerated at the region of the final rupture of the two daughter animals. This regenerated portion of the wall was quite convoluted and irregular in outline.

The other individual (fig. 5, *y*) resulting from the splitting of Actinia *A* was very similar in structure to *x*. The right half of this animal, being derived directly from that of the original individual, was entirely normal in structure. The missing half of each pair of directives had been formed, as well as two pairs of non-directives. One mesentery of each of two other pairs of non-directives was complete and their mates were partially so. A single fundament of another non-directive had appeared on the regenerated tissue of the ruptured portion; but no trace of its mate, nor of any of the incomplete mesenteries, could be recognized. As before, the regenerated tissue was irregular in outline and thicker than the neighboring portions of the column wall.

The other double actinian, *B*, (fig. 2) did not have the mouth entirely separated into two when found, as had actinia *A*, neither did it entirely divide into two individuals during the four weeks

Fig. 2 Drawing of Actinia *B*, oral view, showing tri-glyphic mouth.

Fig. 3 Diagram of mesenteries as seen through oral disc of *A* at time of first observation.

Fig. 4 Diagram of mesenteries as seen through oral disc of *A* just before the final step in the longitudinal fission occurred.

Fig. 5 Section through the two daughter animals resulting from the division of *A*, showing relative positions of the mesenteries.

Fig. 6 Diagram of mesenteries as seen through oral disc of *B* at time of first observation.

Fig. 7 Section through *B* just below the oral disc, showing the relative positions of the mesenteries four weeks after figure 6 was made. Upper ends of two esophageal tubes entirely separate.

Fig. 8 Section through *B* at lower end of esophageal tubes, showing common opening of mouths into gastro-vascular cavity. A double set of siphonoglyphs, however, is present.

and two days during which it was under observation. It however did complete the formation of two separate diglyphic mouths, thus giving further evidence of the progressive nature of the development of double forms of A. bermudensis.

When found, the mouth was triglyphic, i.e., it was roughly triangular in shape, with the basal side deeply indented, and having a siphonoglyph at each angle. The tentacles were one hundred and thirty-one in number when the animal was found, and had increased to one hundred and fifty-three when it was killed and dissected. The complete, and many of the incomplete, mesenteries (fig. 6) could be seen through the transparent oral disc. From two of the siphonoglyphs extended paired directives, while from the third or 'apical' one there appeared to be three mesenteries extending to the column wall. Between the divided siphonoglyphs were two non-directives, one to each half of the mouth (fig. 6). Between the siphonoglyphs at the basal angles of the roughly triangular mouth, and the one at the apex, there were five pairs of non-directives on each side of the animal.[3] Between the adjacent pair of complete mesenteries could be seen traces of from one to three pairs of incomplete septa (only one pair is indicated in the diagram). In all, twenty-four paired and five unpaired complete mesenteries were present.

By the end of the fourth week of observation, the animal had formed two entirely separate, diglyphic mouths, and had increased the number of tentacles to one hundred and fifty-three. As want of time did not permit further observation, the animal was killed and dissected. The two mouth openings were entirely separate, but the two oesophageal tubes were not so, being united at their extreme buccal ends, and opening into the gastrovascular cavity through a common orifice; the four siphono-

[3] Figure 6 reproduces the conditions as they were observed in the living animal through the thin-walled oral disc, and represents the right half of the animal as containing 6, instead of 5, complete non-directive pairs of mesenteries. Sections of the later stage of this animal made subsequently (figs. 7 and 8) show that a mistake was probably made in sketching the mesenteries of the right side; for in this later stage only 5 such mesenteries are to be found; the possibility of error in counting the mesenteries in the living condition is considerable, but not so in the sections.

glyphs, however, maintaining their individual identity along the whole length of the esophagus. No evidences of any mesentery connecting the upper ends of the double tube, such as those recorded by Parker ('99) and by Torrey and Mery ('04) for Metridium, were found.

A transverse section of the column (fig. 7) just below the oral disc showed the following arrangement of the mesenteries. There were paired directives to each of the four siphonoglyphs, and the right and left halves of the animal, outside of the siphonoglyphs, being the right and left halves of the original parent animal, remained unchanged, each having five pairs of non-directives with two or three pairs of incomplete septa between each pair of the complete mesenteries. Between the two more widely separated siphonoglyphs were two pairs of non-directives, one pair to each esophagus, and between these pairs and the adjacent pairs of directives were the fundaments, in one case of one pair, in the other, of two pairs of mesenteries whose destiny whether development into complete or incomplete mesenteries could not as yet be foretold. Between the siphonoglyphs of the other end of the double oesophagus were the fundaments of but one pair of mesenteries.

A transverse section of actinia *B* at the level of the opening of the oesophagus into the gastrovascualr cavity (fig. 8) showed practically the same arrangement of the mesenteries as in figure 7, except that there were no signs of any intermediate septa between the two pairs of directives of the sister siphonoglyphs.

As is evident from the above description, the planes of separation of the animals were parallel to the long axes of the original mouths. Each siphonoglyph was halved and apparently one directive of each pair also went to each daughter half, making the division plane fall in entocoelic spaces in both cases. This agrees with the results as shown in Metridium, where division was always either entoceolic or ectocoelic, never half one and half the other.

The evidence also seems to point to the fact that, in Actinia bermudensis at least, longitudinal fission does exist, and that double-headed and double-mouthed specimens may be stages in

its progress. The primary steps are very slow, but in the case in which the process was watched to its completion, the last stages were extremely rapid and were accompanied by rupture, followed by subsequent regeneration of the torn material in each of the daughter animals. These resultant animals resembled normal actinians in size, shape, and the general number and arrangement of the mesenteries. The tentacles were slightly fewer in number than are those of the normal specimens of equal size.

When found the double animal *A* was full of embryos of all sizes, from the ciliated planula to young with twelve tentacles, and it set free an occasional one up to a week before the completion of the fission process. On the dissection of the two daughter animals, a few small planulae were dislodged from among the coils of the acontia. The presence of young was another proof of the normal condition of the animals during fission, which probably also was of natural origin. This similarity between the resultants of such fission and normal animals is paralleled by the cases of 'circumstantial' daughter pairs of Metridium found by Parker ('99), which could not be distinguished from thousands of other normal individuals except by their peculiar isolated positions.

Though neither the source nor the cause of these double forms of Actinia has been ascertained, it seems to me certain that they could not have resulted from double embryos, and even if the condition began with an injury, the later events show that they ended in a slow but actual process of longitudinal fission, and that they were not permanent forms incapable of further development. It also seems probable that the animals resulting from such a fission are in all respects similar to specimens arising through the process of normal development from the egg.

Harvard University, October 1, 1917.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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ON THE ORIGIN, NATURE, AND FUNCTION OF THE CRYSTALLINE STYLE OF LAMELLIBRANCHS[1]

THURLOW C. NELSON

From the Zoological Laboratory of the University of Wisconsin

SEVENTEEN FIGURES

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1. INTRODUCTION

"What has not been written concerning the crystalline style, and in how many ways has not the mind of man sought to understand its true meaning, and to get to the bottom of its nature!"[2]

With these words a recent investigator (Matthias, '14) sums up well the history of the crystalline style of the lamellibranchs. Few molluscan structures have excited more interest among

[1] Submitted as thesis for the degree of Doctor of Philosophy, June, 1917.

[2] Translation is mine.

scientists than this singular organ, and yet, where described in many of the modern text-books, its function is ignored or held more or less doubtful. It seems that we really know little more regarding the biological rôle of the crystalline style than we did two centuries ago. A survey of the literature shows that the conclusions of the many investigators are decidedly contradictory. Much of the evidence advanced is not well supported and the conclusions in many instances are mere guesses.

It is to be noted, furthermore, that some recent authors (von Fürth, '03; Gutheil, '11; Matthias, '14; Allen, '14, and others) have accepted the work of Mitra ('01) as conclusive. The investigations of this scientist were comprehensive and carefully conceived as far as they were carried out. However, as we shall see later, much of the credit given to Mitra rightfully belongs to earlier workers. He gives no survey of the literature beyond the theories presented in four text-books, and some of his conclusions are untenable in the light of more recent investigations.

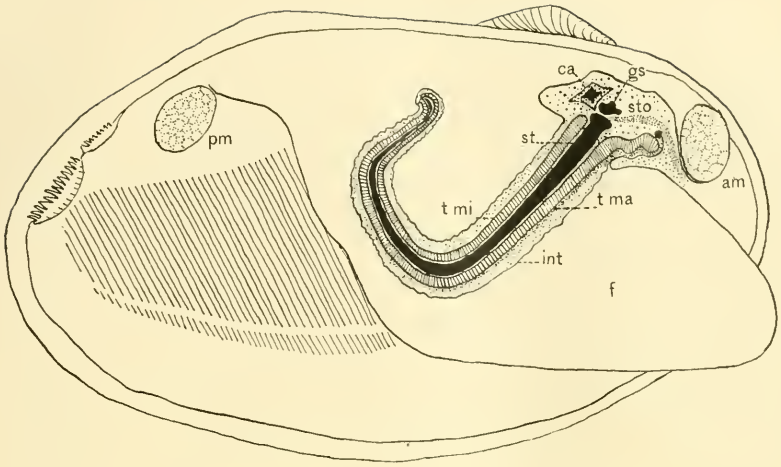
The present contribution is an attempt to bring all known data under one head and to attack the problem from every possible angle.

My own investigations, together with such results of other workers as are applicable, will be considered under the main topics of morphology, histology, physiology, embryology, ecology, and evolution.

The crystalline style

In figure 1 is shown a fresh-water mussel, Anodonta grandis, dissected to show the crystalline style and the part of the alimentary canal in which it is lodged. As these structures as found in this species are fairly typical of the more common lamellibranchs, Anodonta will be used as the type form.

The intestine leaves the posterior end of the stomach and runs in a posteroventral direction along the base of the foot to within a short distance of the posterior margin. Here it turns dorsad and, making a wide bend, continues dorso-anteriorly through the hepato-pancreas to a point below the anterior end of the heart. Here the intestine makes a backward turn upon itself, and after



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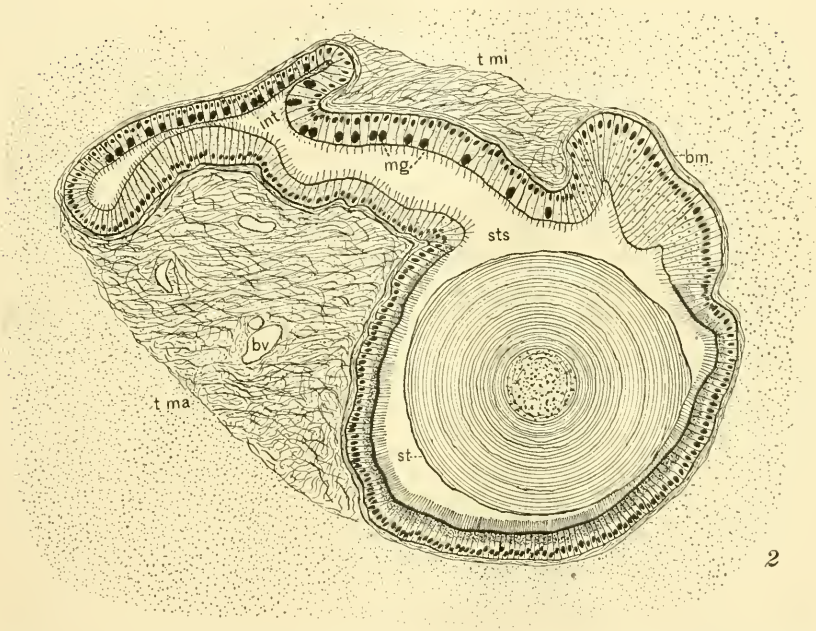

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Fig. 1 Anodonta grandis; dissection from right side. *am*, anterior adductor muscle; *ca*, opening into dorsal caecum of stomach; *f*, foot; *gs*, gastric shield; *int*, intestine; *pm*, posterior adductor muscle; *st*, crystalline style; *sto*, stomach; *t ma*, major typhlosole; *t mi*, minor typhlosole.

Fig. 2 Lampsilis anodontoides; transverse section through style sac and intestine near anterior end. *bm*, basal membrane; *bv*, blood-vessel; *int*, intestine; *mg*, mucous gland; *st*, crystalline style; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

three more loops in the visceral mass passes to the rectum and anus.

The first portion of the intestine (the 'Magendarm' of the Germans) which lies between the stomach and the sharp bend beneath the heart, consists essentially of two regions. On the right side is the intestine proper and on the left the groove which lodges the style. Since this groove or tube is open at both ends, in the type under consideration, I shall refer to it as the style sac, rather than as the style caecum, as it is commonly called.

Incompletely separating the intestine and style sac are a dorsal and a ventral ridge, forming two 'typhlosoles' which extend throughout this portion of the intestine. The broad surfaces of these ridges are in apposition, leaving a narrow slit through which the style sac and intestine are in communication throughout their extent. The relation between these structures is better understood from figure 2, a transverse section through this portion of the alimentary tract.

The style itself, when fully formed, completely fills the lumen in which it lies. It tapers gradually from before backwards, becoming a more tenuous thread at the posterior end of the style sac. The anterior end projects from the pylorus across the cavity of the stomach to the opposite wall.

At the point of contact between the head of the style and the gastric mucosa there is developed a plate-like structure of cartilaginous consistency. It conforms to the outline of the stomach wall at that point, and may bear an apical projection near its center. In some forms (Pholas, Donax, Martesia) this cartilaginous sheet lines the whole of the stomach cavity. As this structure has not, to my knowledge, been described by English or American investigators, I propose the name 'gastric shield.'

To avoid any misunderstanding, there is introduced here a synonomy of terms used by the earlier investigators. The crystalline style of English-speaking scientists is the 'stylet cristallin,' of Barrois ('89), 'tige hyalin,' of Siebold ('48), 'tige cristalline,' of Coupin ('00), the 'Dünndarmkörper' of Hazay ('81), and the 'stylus cristallinus' of its discoverer, Anton de Heide (1686).

The gastric shield is represented by the 'Cristallgriffin,' of Bojanus ('19), 'Knorpelstiel,' of Hazay ('81), 'dreizackige Pfeil of Jordan ('13), 'fleche tricuspide,' or 'sagitta tricuspis,' of Poli (1791), and the 'stomachal cuticle' in Pelseneer's volume on the Mollusca in Lankester's Treatise.

Historical

A complete chronological review of the literature dealing with the crystalline style would be beyond the limits of the present paper. A comprehensive digest of all former investigations and theories is, however, necessary. I shall, therefore, sum up most of these data, not chronologically, but group the various authors according as their conclusions favor one theory or another.

The following account is based chiefly upon the historical summaries found in the works of Barrois ('89, '90) and List ('02). Wherever discrepancies occur between the accounts as given here and for all of the important works, reference has been made to the original articles.

Anton de Heide (1686), in his monograph of the common mussel, describes the 'stylus cristallinus.' He refers to the work of Willis on the oyster, six years previous, in which he described a structure analogous with the 'spinali medullae' of the vertebrates. From the text it is probable that Willis referred not to the style, but to the typhlosole separating the style sac and true intestine. We are therefore justified in recognizing de Heide as the discoverer of the crystalline style. Its function was ignored by this investigator who, however, hazarded the guess that it might be an alimentary ferment or perform some rôle in the act of generation.

Lister (1696), in his anatomy of bivalve molluscs, pictures the stylus of Pectunclus vulgaris and Mytilus marinus (edulis), but gives no appreciation of the structure or usage of them.

Swammerdam (1737) refers to the presence of the style in Mytulus belgicus, evidently a fresh-water species.

Lesser (1744) reviews the literature to date, but adds no contributions of his own.

The first comprehensive investigation of the nature and function of the crystalline style was made by Poli (1791–95). He describes accurately the appearance of the style and its relations to the rest of the alimentary system. He was the first to note the presence of the gastric shield, which he likened to a cartilaginous three-pointed arrow, 'sagitta tricuspis,' borne on the head of the style.

Investigating the physical and chemical properties of the style, he found it to be soluble in water and coagulated by boiling water and alcohol. He believed that it served in some way to regulate the flow of 'bile' from the biliary crypts. Cuvier (1805) adds nothing new, but admits the conclusions of Poli.

From this point on investigators are so numerous that it is best to classify them according to the theory which they supported.

A. *An organ of support.* From its position in the alimentary tract, Carus, (1818), believed it to be a vestige of a splanchnic skeleton, homologous with the teeth of the sea-urchin. Garner ('41) believed its function was to give rigidity to the foot. DeBlainville ('48) claimed the simultaneous existence of several styles arising in the sinuses of the biliary canals. He had nothing to say regarding their use.

B. *Mechanical functions.* Meckel ('29) considered it homologous with the radula of the Cephalophores, and consequently an organ of mastication. Garner ('41) agreed with this interpretation, and thought that the points of the gastric shield served to modify the flow of 'bile' as first held by Poli. Deshayes ('48) gives good figures of the gastric shield which he believed to be important in mastication. Clark ('50) thought that the style acted as a pestle to grind up the alimentary matter.

Huxley ('53) was the first to consider the style an epithelial secretion. He says, regarding the style of Pteroceras,

> The end of the style is opposed by one or two cartilaginous plates (the gastric shield) upon the principal elevation. It seems probable that the style is secreted by the walls of the pyloric caecum, and that it plays the rôle of a gastric plate to aid in grinding up the alimentary matter, though its transparent and delicate structure seems ill fitted to this purpose.

Milne-Edwards ('59) believed that its function was to stir the contents of the stomach during digestion. Hessling in the same year agreed with him that the style was an epithelial secretion. Both evidently were ignorant of the earlier work of Huxley.

Vanstone ('93) thought that the style was homologous with the stomach plates of snails and served to grind up the food.

Sabatier ('77), in his monograph of the common mussel, gives the first exhaustive histological treatise on the style region. He recognized the two ridges, or 'typhlosoles,' incompletely separating the style sac and intestine, and their relation to the style. His study of the heavy ciliation of the wall of the style sac led him to believe that the food of the mussel was caught between the cilia and the style, and mixed and rolled around, the style playing the part of an organ of mastication. He considered that the epithelium of the style sac served for the absorption of dissolved matter. He did not suspect the real relation between the style and the secretory epithelium which he describes.

C. *An aid to absorption.* Krunkenberg ('86) considered the style to have a function similar to that of the typhlosole of Lumbricus, pressing the alimentary matter against the absorbing epithelium. Von Fürth ('03) agrees with this interpretation of the anatomical function of the style. Grave ('03) believed that the style of the oyster acts as a plug to prevent the too rapid movement of alimentary materials and to exclude foreign particles of large size from the intestine.

D. *Reserve of nutriment.* Hazay ('81) made detailed observations of the occurrence of the style in the Unionidae, and distinguished the 'Knorpelstiel,' the gastric shield, and a hyaline string, the 'Dünndarmkörper,' or style proper. According to his observations, the gastric shield is very rudimentary in the spring and summer, attaining its greatest size in the autumn. The style is, in his opinion, formed from a gelatinous mass ('Magengallerte') in the stomach, and is pushed back into the intestine where it remains as a reserve of nutriment. This is kept from flowing into the stomach by the presence of the gastric

shield, which forms a sort of valve over the orifice. During the winter this reserve serves to nourish the animal. His conclusions are based on a study of two closely related fresh-water forms.

The presence of such a reserve of nutriment in the intestine was held by Krunkenberg ('86) to be untenable.

The inaugural thesis of Haseloff ('88) is the most extensive single work on the crystalline style. He studied seven different forms, but based his conclusions largely upon Mytilus. After describing the general anatomical relations of the style, the author cites experiments in which Mytili were kept in filtered and in normal sea-water. Finding that the style grew thinner and finally disappeared in those animals which were starved, he concluded that it must be a reserve of nutriment.

Haseloff was not aware of the work of Hazay until after he had finished his investigations. From his own results and those of Hazay, he concluded that the style was a structure built up in time of excess nutrition and absorbed in times of want. As for its origin, he believed it to be a chemical transformation of surplus nutriment, and not an epithelial secretion. Chemical tests applied by him showed the style to be albuminous in nature. He concluded that the presence of the style is almost universal in the lamellibranchs.

Dubois ('92) thought that the style serves as nutriment for parasites useful to their host.

Stempell ('98) believed the style to be lacking in the Nuculidae. The large gastric shield which he found in these forms was held to be a reserve material, absorbed by the gastric epithelium and used in the development of the sexual organs.

List ('02) in his beautiful monograph of Mytilus, gives a most comprehensive treatment of the subject. The historical summary is fairly complete and accurate, save that he does not give proper credit to some former workers. His investigations cover the anatomical, histological, and certain physiological aspects of the problem. He was the first to study the formation of the style in the living animal, and found that color particles taken up by the mussels in feeding were built up into the body of the style.

The nutriment taken into the stomach he believed to be mixed with the style substance, carried to the hepatopancreas and there digested. The course of the color granules was traced into the hepatopancreas, back to the stomach, and thence to the intestine. He believed that the style might serve as a reserve of nutriment in the Unionidae during hibernation, but that in marine forms this nutriment would be superfluous and therefore thrown away. Matthias ('14) in discussing List's idea of superfluous nutriment, considers that the mussels with all their ascribed stupidity are not so utterly 'stumpfsinnig' that they would willingly throw out the nutriment which they had so painstakingly gathered.

E. *Relation to the organs of generation and the process of reproduction.* De Heide (1686) thought the style might serve some purpose in the act of generation, but gave no reason for his belief. Poli (1791) advanced the same theory, also without any evidence in its support. In later years both of these investigators abandoned this interpretation of the function of the style.

After nearly two hundred years this rejected theory was again advocated by Cailliaud ('56). He held the Pholadidae to be hermaphroditic (since disproved) and that the style served some function during fecundation. Carus ('18) believed that the style might have some connection with the sex function.

Hoffmann ('14) following Stempell ('98), claimed to have ascertained a reciprocal relation existing between the gastric shield and the sexual organs. Comparing the mass of the gonad, the gastric shield, and the style, he concluded that the shield was not as well developed in males and females with mature gonads as in a male specimen where the testes were immature.

F. *Serves as a means of transport for the nutriment; lubricating function of the style.* Although the conclusions of Barrois ('89–'90) place his work under this head, it must be said in all fairness that his treatise on the crystalline style of the lamellibranchs is the most comprehensive and accurate contribution to the subject which thus far has appeared. Many subsequent workers have evidently paid but little heed to it, his results

either being ignored or else appropriated without giving proper credit. Much of the value of this work of Barrois lies in the thorough critical analysis of all former work which it contains. The historical summary is quite complete to date of publication. Most of his results will be considered in their proper connection on the following pages, but it may be said in passing that the investigations covered an exhaustive study of many forms, dealing with the anatomy, histology, chemical composition, and the physiological significance of the style and its attendant structures. The weakness of this contribution to the subject lies in Barrois' failure rightly to interpret the facts which were before him. He observed that the style was pushed forward into the stomach, there to be dissolved under the action of the gastric juice. He believed that the resulting viscous mass formed a sort of cement, which surrounding the sand grains and other foreign materials present, encrusted them, and thus prevented injury to the delicate lining of the alimentary canal. The gastric shield was believed by him to have a protective function similar to that of the 'Trichter' of insects.

Pelseneer, in Lankester's Treatise on Zoology ('06) and in his own earlier paper ('91) follows Barrois, and believes that the function of the style is to lubricate the alimentary mass. Schultze ('90) accepts the results of Barrois in their entirety, while Kellogg ('92) disagrees with both Barrois and Pelseneer, since where large amounts of sand are ingested the style could not form a sufficient protective covering. Coupin ('00) thought that the mucus of the style might serve to surround sharp particles and thus protect the epithelium.

G. *A mass of enzyme, or of enzyme and mucus.* Anton de Heide (1686), the discover of the style, hazarded the guess that it might be a mass of digestive fluid, but made no attempt to determine the fact. Wilson ('77) believed the style to be a mass of oesophageal mucus and gastric juice, but did not demonstrate any action of the style substance on food material.

The first attempt to determine the presence of enzymes in the style was made by Coupin ('00). He believed that the style was a mass of mucous substance, saturated with digestive fluid,

and he demonstrated the action of the amylolytic ferments present.

Mitra ('01), with no review of the literature save the theories given in the text-books of Gegenbaur, Balfour, and Claus and Sedgwick, repeated the work done by Barrios, Coupin, and some others, and concluded that the style was a mass of enzyme, in the nature of a globulin. Apparently he did not know of the existence of the gastric shield. His conclusions will be considered in some detail in the discussion of this paper.

Von Fürth ('03) followed Mitra, but believed it more reasonable to suppose as did Coupin ('00), that the style was a mass of mucus saturated with diastases, rather than a solid mass of enzyme. Matthias ('14), whose work has already been considered, admitted the conclusions of Mitra. Allen ('14), accepting the conclusions of Mitra in the main, showed that the formation and dissolution of the style are dependent wholly or in part on the feeding activities.

In addition to the above authors may be listed the following, whose work is of less importance:

Selenka ('68), in the description of the anatomy of Trigonia, notes the presence of the style, lying in a blind sac opening into the stomach. Egger ('87) describes the appearance of the style with its co-axial layers in Jouannetia cumingii. Grobben ('92) gives an account of the nature of the cilia of the intestine and of the style sac. Mention of the style is made by Moquin-Tandon ('85) in the Unionidae, and by Blanchard ('61) in the Pholadidae.

Other investigators and their conclusions, not listed here, will be considered in their appropriate connection in the main body of the present paper.

EXPERIMENTAL

Materials and methods

The investigations reported in this paper were carried on in the Zoological Laboratory of the University of Wisconsin, and at the floating laboratory of the New Jersey Agricultural Station for research in oyster culture, on the coast of New Jersey. The

work with fresh-water forms was done at Madison, using chiefly Anodonta grandis, Lampsilis luteolus, and L. anodontoides. These were collected from the near-by lakes and kept supplied with lake water. The marine species were studied at the summer station at Tuckerton, N. J., which is situated on the natural beds of the oyster, Ostrea virginica. Within a few feet of the laboratory also occurred the hard clam, Venus mercenaria, and the ribbed mussel, Modiolus modiolus. The bulk of the work with salt water forms was confined to these three species.

In studying the formation and physiological significance of the style, dissections were made of the living animals. Care was exercised to open the molluscs immediately after removal from the water, and to dissect open the stomach and intestinal walls in such a manner as to interfere as little as possible with the main nerve commissures. Wherever ciliary action was investigated the animal was kept in a small trough under the binocular, supplied with a constant stream of water at the same temperature as that in which it had been living.

Ciliary currents were traced by the movement of food material, and also by the introduction of carmine grains or fine sand.

Some difficulty was experienced in sectioning the style sac since with ordinary methods of fixation the style becomes so brittle that it invariably crumbles. The best results were obtained by fixing the material in Bouin's fluid, running up through the alcohols as rapidly as possible, and clearing in wintergreen oil, after which the tissue was imbedded in paraffin.

The sections were stained in Heidenhain's iron-hematoxylin and counterstained either with eosin, acid fuchsin, or mucicarmine. Fixation with osmic-acid solutions followed by safranin and gentian violet was very unsatisfactory, due chiefly to the slow action of the fixative on the rather large pieces of tissue necessarily used.

With Bouin's fluid the fixation of the cilia is almost instantaneous and, as a result, the separate cilia stand up clearly and distinctly, instead of being in a confused mass as usually happens during fixation.

2. ORIGIN

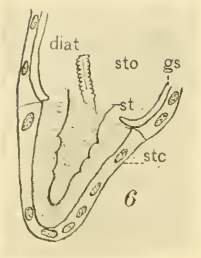
Gross structure of the style-bearing organs

The crystalline style and its attendant structures in Anodonta have been described, p. 56. The condition found in the other groups of the lamellibranchs follows:

The style occurs in its simplest form in the more primitive groups, the Protobranchs and Filibranchs. In Nucula, as an example, the style lies in the intestine itself, the alimentary canal showing no indication of a separation into two tubes. In the Filibranchs are certain transitional forms. Arca, for instance, contains species which, according to Matthias, show the simple condition, as in Nucula, and others in which there is the beginning of the formation of a separate style sac.

In Arca platei the intestine is roughly ovoid in cross section and is nearly filled by the style. At one side the intestinal wall forms a slight groove down which pass the waste materials. In Arca barbata this groove is somewhat deepened and, by the infolding of the intestinal wall, forming two ridges, this furrow is partially separated from the part bearing the style. These infoldings, which represent the typhlosoles of Anodonta in their most primitive condition, are not in apposition in this species, but leave a wide space through which the intestinal groove and the style-bearing portion are in communication (fig. 3).

A condition intermediate between that found in these two species occurs in Modiolaria marmorata where, according to List ('02) only a very slight infolding of the intestinal wall occurs. The epithelium of the alimentary tract is covered with large and powerful cilia, by means of which, on the one hand, the style is pushed forward into the stomach, and on the other, the waste matter is passed outward. By continued growth and differentiation, the epithelial infoldings of the intestine develop broad flat surfaces which come into apposition and separate the intestine from the style sac save for a narrow slit, as found in Anodonta (fig. 2). Coincident with these changes to form the typhlosoles, the epithelium of the style-bearing region becomes thrown up into a series of transverse folds, extending throughout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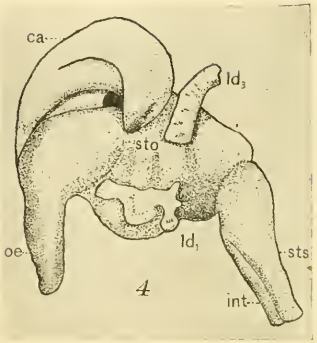
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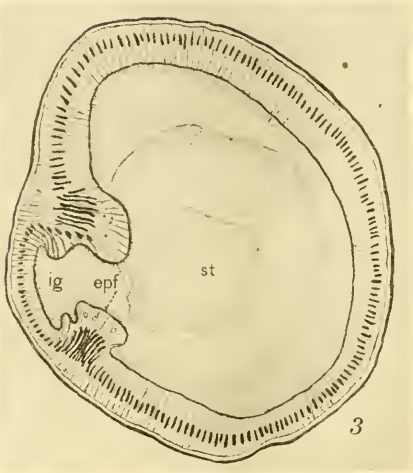
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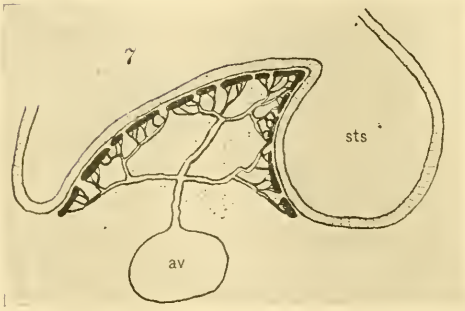
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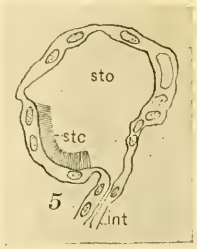
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the length of the style sac, forming a series of 'bearings' upon which the style rests (figs. 9, 10).

The typhlosoles vary in size and position according to the species. In the forms examined by me they are dorsal and ventral, and thus separate the alimentary canal into right and left halves as far as the end of the style sac. In all these species I have examined, the ventral typhlosole is the larger. As a result of the bending of the alimentary canal the typhlosole, which is dorsal at the anterior end, becomes ventral throughout the posterior half of the style sac. I shall, therefore, speak of the typhlosoles as major and minor, rather than as dorsal and ventral.

These structures gradually diminish in size from before backward. In an adult Anodonta, 14.5 cm. long, the major typhlosole measures about 3 mm. across at the anterior end, narrowing down to 2.5 mm. at the posterior end of the sac. The minor typhlosole measures 2 mm. and 1 mm., respectively.

In the species which have just been considered, where the style caecum and intestine are completely separated, the two tubes merge into one at the end of the style-bearing region. Where the separation is more nearly complete, as in some of the Unionidae a small diverticulum occurs at the end of the style sac at the point where the intestine makes the sharp backward

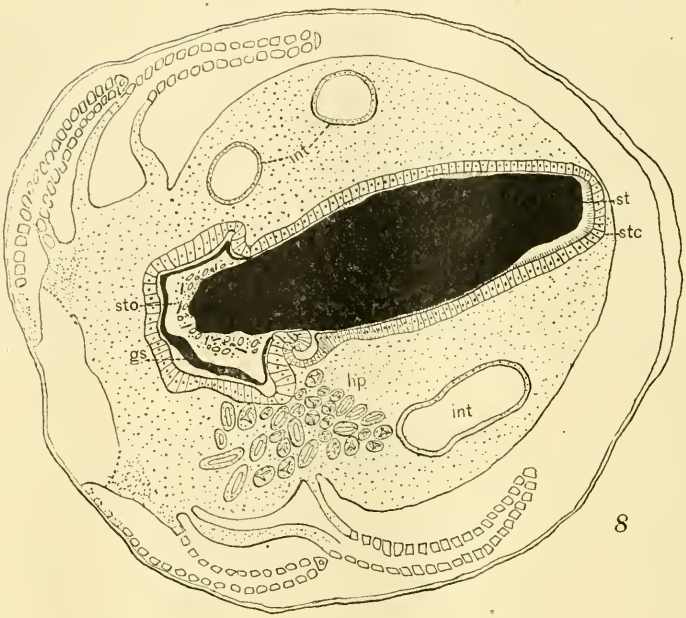
Fig. 3 Arca barbata; transverse section through alimentary canal posterior to the stomach (Matthias, fig. 17). *epf*, epithelial folds; *ig*, intestinal groove; *st*, crystalline style.

Fig. 4 Anodonta cellensis; plaster-of-Paris mold of stomach, oesophagus, and anterior portion of intestine and style sac (Gutheil, fig. 3). *ca*, dorsal caecum; *int*, intestine; ld_1, ld_3, left and dorsal ducts of hepato-pancreas; *sto*, stomach; *sts*, style sac.

Fig. 5 Dreissensia polymorpha; sagittal section through stomach, intestine, and anlage of the style caecum (Meisenheimer, fig. 132). *int*, intestine, *stc*, wall of somach which evaginates to form the caecum of the crystalline style; *sto*, stomach.

Fig. 6 Dreissensia polymorpha; longitudinal section through the style caecum (Meisenheimer, fig. 133). *diat*, diatoms; *gs*, gastric shield; *st*, crystalline style; *stc*, caecum of the crystalline style; *sto*, stomach.

Fig. 7 Anodonta cellensis; transverse section through the major typhlosole and style sac showing distribution of blood-vessels (Schwanecke, fig. 24 a, somewhat diagrammatic). *av*, visceral artery; *sts*, style sac.



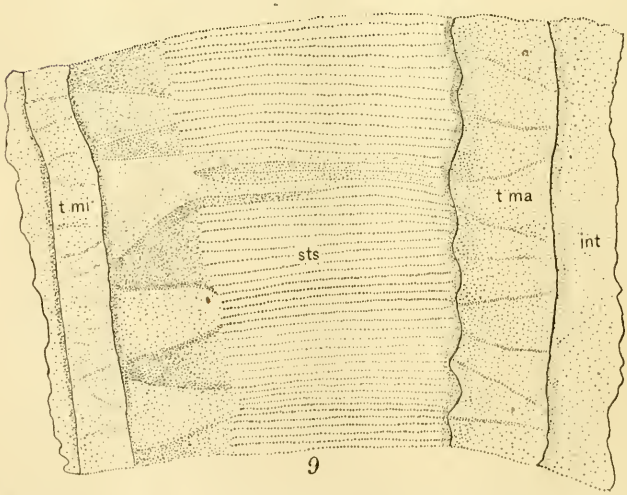


Fig. 8 Martesia; median transverse section of entire body. *gs*, gastric shield; *hp*, hepatopancreas; *int*, intestine; *st*, crystalline style; *stc*, caecum of the crystalline style; *sto*, stomach.

Fig. 9 Anodonta grandis; enlarged view of the surface of the typhlosoles, intestine, and style sac, opened from right side. *int*, intestine; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

bend beneath the heart. In some forms (e.g., Mytilus galloprovincialis, according to List, '01) this diverticulum is prolonged a short distance posteriorly as a blind sac distinct from the intestine.

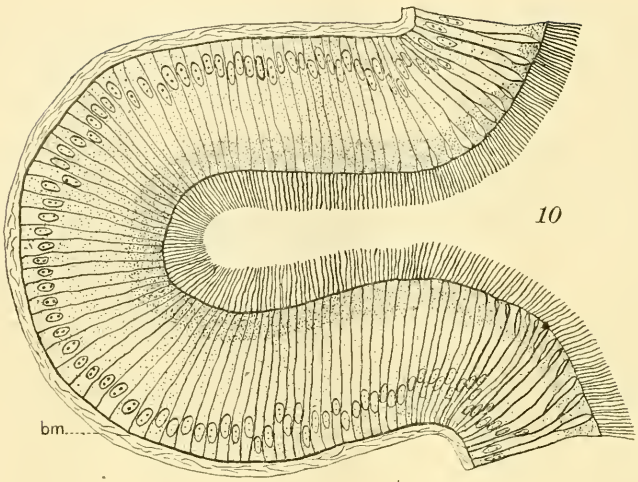
The style sac in certain of the higher lamellibranchs has become completely cut off from the intestine, forming thus a distinct caecum. This is entirely independent of the intestine, and opens into the stomach by a separate orifice. Such a condition is found in Martesia (fig. 5).

As has been pointed out by Matthias ('14), there exist, therefore, three distinct types of lamellibranchs as regards the position of the style, with transitional forms making a graded series from one to the other. First, those species in which the intestine itself bears the style; second, those in which the style sac and intestine form two tubes, incompletely separated by the typhlosoles, and finally the forms in which the style sac exists as a diverticulum distinct from the intestine proper.

The epithelium around the opening of the style sac into the stomach is raised into a ring which forms a sort of 'bushing' about the style. When the latter is fully formed it fits this orifice so snugly that no particles from the stomach could possibly pass between the style and the wall of the sheath, even if the effective stroke of the cilia were in such a direction as to permit it.

In the forms where the intestine and style sac are incompletely separated by the typhlosoles, the major typhlosole extends anteriorly into the stomach, forming a sort of tubercle as in Anodonta, or a long ridge on the ventral wall of the stomach as in Modiolus (fig. 12). Other lamellibranchs examined have shown a somewhat similar prolongation of the major typhlosole.

The stomach presents the most diverse modifications in the different species. In general it is an elongated, oval sac, the lateral walls of which are more or less compressed. Various outpocketings of the epithelium are present, usually in connection with the orifices of the hepatic ducts. The number of these varies, according to Pelseneer, from three in certain of the Nuculidae, to as many as twelve in Mytilus. Three are present in Anodonta (fig. 4).



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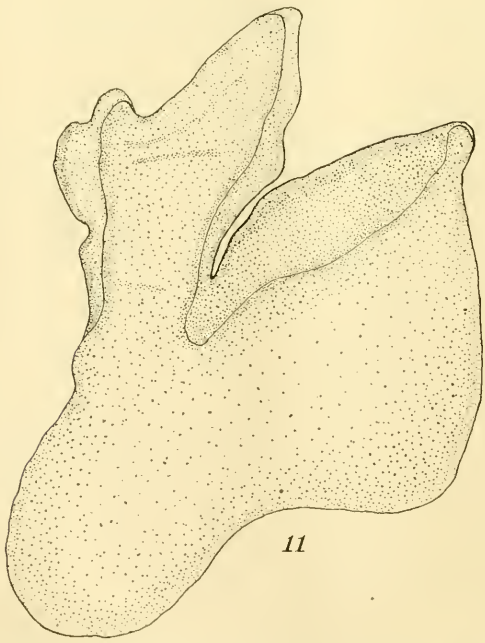

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Fig. 10 Lampsilis anodontoides; longitudinal section of the wall of the style sac, showing one of the transverse furrows. *bm*, basal membrane.

Fig. 11 Modiolus modiolus; the gastric shield, concave aspect.

The epithelium of the stomach is thrown into many folds and ridges, with deep grooves passing between them. Figure 16 gives the appearance of the stomach of Anodonta when opened along the right margin of the dorsal wall, and spread out flat. It also shows the relation of the two orifices, opening side by side and separated by the typhlosole, and the large deep groove leading across the wall of the stomach and entering the intestine proper.

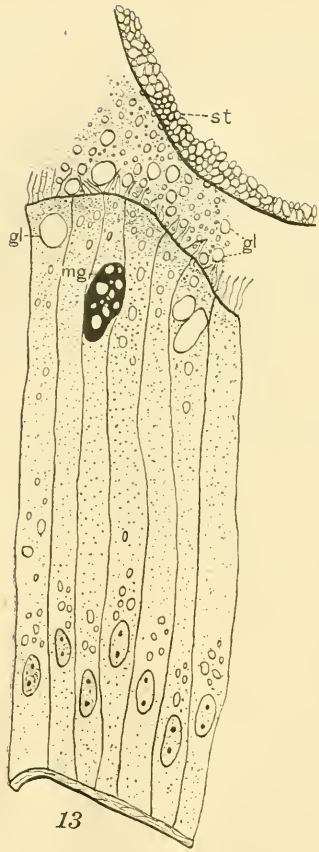
In some forms a portion of the stomach is more or less separated by a constriction, forming, in Anodonta, a pouch-like diverticulum on the dorsal side (fig. 1) or a distinct caecum extending the length of the stomach, beneath its ventral wall, as in Modiolus. This blind sac performs in the latter genus, a very important function, as will be shown later.

The gastric shield

On the anterior wall of the stomach, at the point where the style comes in contact with the mucosa is a singular structure which I have called the gastric shield. This is a thin, plate-like sheet, as clear as glass, and of the consistency of cartilage. In an adult Anodonta it measures approximately 8 x 5.5 mm. in its greatest extent. It assumes many diverse forms in different species, but in general I find three main lobes, and a blunt apical projection (fig. 11). The edges of these lobes are firmly held between the folds of the epithelium, and, in some species at least, the border of the shield dips down into the crypts of the hepatic ducts. In some instances a sharp spur of the shield may run for some distance into the orifice.

The cells beneath the shield are, according to Gutheil ('11) the only columnar cells of the entire alimentary tract which are devoid of cilia. Their function is to secrete droplets of a colorless matter which harden to form the shield.

This structure is closely applied to the epithelium lying beneath it, and, as it rests between folds of the stomach wall, it presents an outer surface concave in outline. In Venus an outward projection of the epithelium forms a sort of tubercle which



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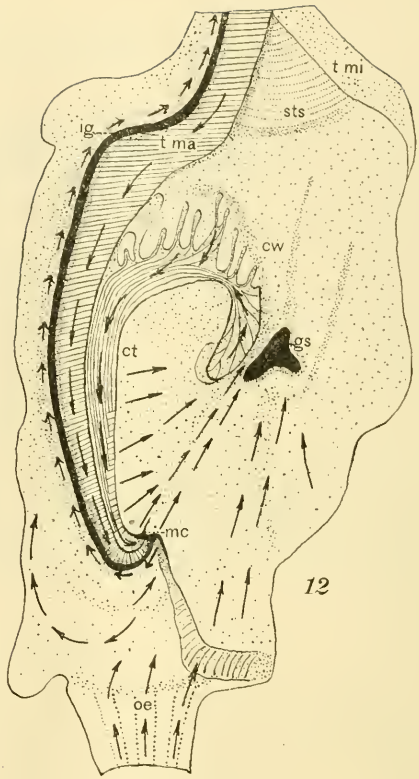

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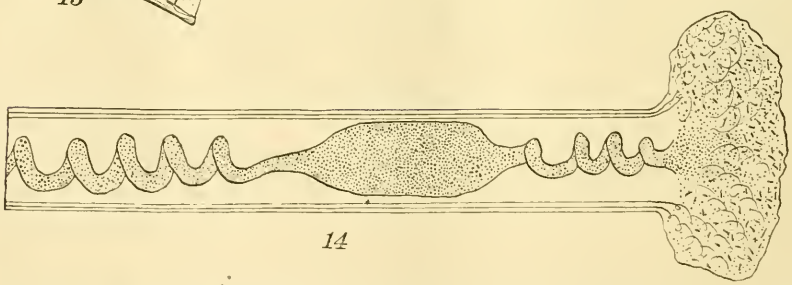

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Fig. 12 Modiolus modiolus; surface of inner wall of the stomach opened from the right side; long arrows indicate direction of ciliary currents on the ridges and exposed surfaces of the mucosa, short arrows show direction in the furrows and grooves. *ct*, ciliated tract leading to mouth of caecum; *cw*, ciliated whorl from which this tract originates; *gs*, gastric shield; *ig*, intestinal groove; *mc*, mouth of caecum; *oe*, oesophagus; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor, typhlosole.

Fig. 13 Lampsilis anodontoides; enlarged view of the secreting cells of the dorsal wall of the style sac. *gl*, globules of secreted material; *mg*, mucous gland; *st*, style.

Fig. 14 Anodonta grandis; optical section of freshly removed style showing inner spiral core of food materials, and the solution of the head of the style.

acts as a brace for the shield. In Ostrea the smallest of the three lobes is concave on its upper surface, forming a bowl-like depression.

In Donax, according to Barrois, the gastric shield covers nearly the entire wall of the stomach. At the crypts of the hepatopancreas it dips down into the orifices and lines them. Martesia (fig. 7) exhibits the same type of shield.

In all cases which I have examined the shield bears at its apex a blunt projection of much heavier nature than the lobes themselves. Under natural conditions the head of the style is directed against this projection, while the food mass around the style is in contact with the lateral lobes of the shield. The significance of this will appear later.

Nervous and vascular connections of the style sac

According to Schwanecke ('13), blood is supplied to the style sac and other parts of the alimentary canal through the visceral artery, a large branch which leaves the anterior aorta just ventrad the stomach, and courses posteriorly to the upper part of the intestine and style sac. Here it divides to form two large trunks, which in turn supply the greater part of the alimentary canal. Most of the blood going to the intestine is carried to the typhlosoles. Here there are no true capillaries but the smaller arteries form large lacunae beneath the epithelium. As a result, an almost continuous haemal cavity is formed in close conjunction with the secretory and ciliated cells (fig. 7).

The venous system is less well defined than the arterial. The blood from the lacunae is collected by several large veins and carried to the sinus venosus.

The lamellibranchs, according to Pelseneer (Lankester's Treatise), have no differentiated stomatogastric nervous system. Nerve strands to the alimentary canal are given off from the median faces of the two branches of the cerebrovisceral commissure. More recently, Splittstoeszer ('13), reviewing the work of Keber and Duvernoy on the nervous system of molluscs, has presented a careful and extensive investigation of the nervous

system of Anodonta cellensis. According to him, three gastric nerves arise from the cerebrovisceral commissures of each side, just posterior to the cerebral ganglia. These send branches to the oesophagus, but principally supply the wall of the stomach. As a result of the intercrossing of many nerve fibers there is formed here a 'solar plexus' from which fibers extend downwards to the wall of the intestine and style sac. This latter region is, therefore, in close nervous connection with the anterior centers of control.

Histology of the style sac and accompanying organs

By far the most extensive and careful part of all former work on the crystalline style has been concerned with the structure of the cells of this region. When one sees the long ciliated cells, with their large distinct nuclei, besides the several types of secreting cells, it does not seem strange that so many investigators, setting out to study the style in all its relations, should have been sidetracked by the beautiful histological material before them. Inasmuch as the present problem lies rather in the realm of physiology than that of histology, I shall consider here only such details of cell structure as are necessary to understand the mechanism for the secretion and movement of the style. For a more complete account of the histology of the alimentary canal of lamellibranchs the reader is referred to the works of Sabatier ('77), Barrois ('89), List ('02), and Gutheil ('11). An extensive treatise on the structure of ciliated cells, and the mechanism of ciliary action with special reference to the typhlosole of Anodonta, will be found in the work of Erhard ('10).

Figure 2 is a transverse section of the style sac and intestine of Lampsilis anodontoides, showing the typhlosoles incompletely separating the two tubes. The typhlosoles are highly vascular, and are composed of loose connective tissue lying between the lacunae or haemal spaces. Very few nuclei occur here. The epithelium of this region is composed of a single layer of long ciliated cells resting on a basal membrane. The cytoplasm is usually filled with many refractive granules and stains rather

lightly. The nucleus is very large, is situated proximally and may contain one or two nucleoli. Vacuoles or granules of absorbed food material often occur in the cytoplasm. From the distal end of each cell project a number of large cilia, the basal fibers of which extend down some distance into the cytoplasm.

Beneath the basal membrane are bands of musculature in the carnivorous Septibranchs, but in the other lamellibranchs, as pointed out by Gutheil ('11), ciliary action has entirely replaced peristalsis, hence muscular elements in this region are lacking.

Lying between the ciliated cells are two other types, namely, secreting cells and mucous glands. The secretory cells are of long goblet-cell type, and when filled with secretion are greatly distended. They occur most numerously in the minor typhlosole.

The mucous glands are large unicellular structures which take a deep red color with mucicarmine, and are sharply differentiated from all surrounding cells. The nucleus lies near the base, and when the upper portion of the cell becomes filled with mucus it is so distended as to appear ovoid or nearly spherical in shape (fig. 13). These glands in Lampsilis are confined to the minor typhlosole and the intestine. I have never found them in this species in the major typhlosole or the style sac (fig. 2).

Where the minor typhlosole joins the style sac there is a striking modification of the epithelium. The surface of the cells dips down to form a furrow which extends parallel to the typhlosole. The cells bordering this groove are twice as long as the other cells of the alimentary canal, are very narrow, and have large, densely staining nuclei. Their distal ends bear few and small cilia (fig. 13). In favorable material great numbers of globules of a substance staining like the style are found in and between the cells. In figure 14 many of these globules are shown beneath the epithelium. At the surface some of these drops have coalesced to form a mass which cements the style to the epithelium at this point.

The epithelium of the rest of the style sac differs markedly from that of the above region in that it is composed of shorter and thicker cells. The nuclei are large, rich in chromatin, and

lie close to the basal membrane. Most striking of all are the great bristle-like cilia on these cells. Of such size are they that one gains the impression that the very ends of the cells themselves have been drawn out into the fiber. The distal portion of the cells is very rich in protoplasmic material which stains deeply, and forms a distinct region extending from the groove on the dorsal side, entirely around the style sac to the edge of the major typhlosole (fig. 2). A better conception of the cilia on these cells may be gained from figure 10, which shows a section through the style sac at right angles to the transverse folds.

The only cells of the epithelium of the stomach which need concern us are, first, those on the folds of the walls and, second, the cells which lie beneath the gastric shield. The former, according to Sabatier ('77), are much more heavily ciliated than the surrounding furrows. The latter, following Gutheil ('11), are the only columnar cells of the entire alimentary tract which are devoid of cilia. Their function is to secrete globules of a clear fluid which hardens to form the gastric shield.

The ciliary mechanism

Nowhere in the animal kingdom is ciliary activity brought to the high degree of development and complexity that it attains in some of the molluscs. Not only has it replaced muscular peristalsis in many of these forms, but, as is shown in the excellent works of Allen ('14) and Kellogg ('15), the lamellibranchs have developed very efficient and delicately balanced modes of feeding, entirely by the use of cilia.

Allen found that the quality of the food permitted to enter the mouth is controlled by bringing into action one or the other of two opposed sets of cilia. These are situated on opposite faces of the transverse folds of the palps. According as these folds lie in their normal position or are erected by reflex muscular action, the food matter, entangled in mucus, is directed into or away from the mouth. By means of this mechanism, streams of food material which contain too large a percentage of foreign particles are directed backwards, and enter the re-

current ciliary channels leading to the base of the incurrent siphon, whence they are expelled. The mouth is a slit-like aperture situated between the palps. It is provided with muscles by means of which it may be closed in case any objectionable matter gets by the palps. So far as I know, no one has followed the ciliary mechanism beyond this point.

The oesophagus is a thin-walled tube, dorsoventrally compressed. Its inner surface is furrowed by a number of distinct longitudinal grooves, one of which is very much larger than the others. Where the oesophagus joins the stomach the epithelium is raised to form a distinct ridge encircling the orifice; in Lampsilis this ridge forms a sort of shelf on the ventral side, which projects into the stomach, nearly to the gastric shield.

All of the longitudinal grooves of the oesophagus end at the stomach except the large one, which extends across the left side of the ventral wall of the stomach to the base of the gastric shield. The cilia in the oesophagus carry the streams of food material up to the shelf-like projection in its ventral wall and also up the large furrow to the base of the gastric shield.

The lateral walls of the stomach in proximity to the gastric shield bear tufts of very powerful cilia. When the walls are in their normal position the effective stroke of these is such as to set into rotary motion any material in contact with them. This rotation is in a clockwise direction when viewing the animal from the anterior end.

All ridges and furrows of the stomach wall, except the region beneath the gastric shield, bear strong cilia which keep up constant currents within the stomach. In general these may be resolved into two main groups: first, those currents on the ridges and raised parts of the gastric epithelium, together with all streams from the oesophagus, which lead to the region around the gastric shield; second, those of the deeper grooves and furrows of the stomach wall which, save for exceptions to be noted later, lead into the large furrow which passes to the right of the major typhlosole and forms the intestine proper.

A detailed description of the comparative anatomy of the stomach and the ciliary mechanisms in each of the lamelli-

branchs studied would lead too far afield. With minor modifications the figure and description of the stomach of Anodonta serves equally well for Lampsilis, Ostrea, and Venus.

In Modiolus, however, there is an important accessory structure of the stomach which is closely connected with one of the functions of the style in this form. That part of the ventral wall of the stomach which lies between the orifice of the style sac and the base of the gastric shield forms a spirally shaped structure suggesting a whorl of a small shell. Close examination proves this to be a series of conical orifices, each connected with a deep groove (fig. 12). These furrows, at first separate, reunite to form a tract which crosses the ventral wall of the stomach to the left of the extended major typhlosole, and enters the ventral diverticulum or blind sac mentioned above (p. 71). The surface of the conical orifices and the furrows leading from them bear large and powerful cilia, which beat in the direction indicated by the arrows in figure 12. The tract formed by the union of these separate channels bears regular transverse furrows which lead to a deep groove on the left side of the major typhlosole. The same structures are continued down into the caecum, where the transverse furrows become somewhat longer.

Figure 15 illustrates the appearance of the caecum when cut along the dorsal wall and opened out flat. The major typhlosole, *T*, passes to the further end of the caecum, where it ends blindly. In apposition to it under normal conditions is a similar ridge, *T'*, while between them the epithelium is thrown up into regular ridges and furrows.

The cilia on the surface of the typhlosole, as well as those on the crests of the transverse ridges, beat inward toward the end of the caecum. This holds true for the region *R'-T-R* of figure 15, while in the region *R-T'-R'* the cilia on these same structures beat in the opposite direction.

In the transverse furrows the cilia are very large and powerful and beat toward the deep groove which follows the base of the major typhlosole and its opposing ridge as shown by the arrows in figure 15. As a result of this mechanism, a particle of debris falling into the transverse furrows at any point would be car-

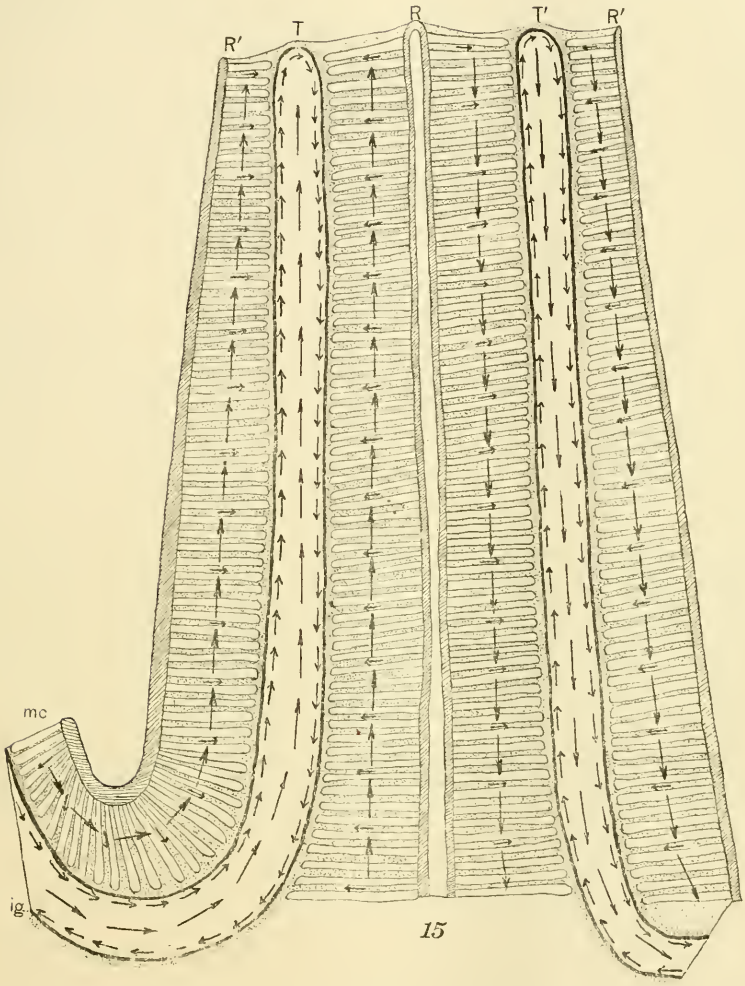


Fig. 15 Modiolus modiolus; food sorting caecum opened from the dorsal side, along the ridge R'; long arrows indicate course of currents on ridges, short arrows show direction in furrows; *R,R'*, narrow ridges; *T*, extension of major typhlosole; *T'*, broad ridge apposing typhlosole in life; *mc*, mouth of caecum; *ig*, intestinal groove.

ried into the deep furrow and thence to the intestinal groove. In this groove, or the intestine proper, the effective stroke of the cilia is toward the posterior end of the body, and thus the waste matter is carried to the outside.

On the broad surfaces of the typhlosoles the effective stroke of the cilia is inward toward the style sac, and somewhat in a posterior direction as well. The cilia on the faces of the two typhlosoles are not equally developed in all species. In Lampsilis I find that those on the major typhlosole are of about twice the length of those on the minor.

The regular transverse folds of the style sac, with their great bristle-like cilia, have been described (p. 65). The effective stroke of these is such as to put the style in rotation in a clockwise direction, and at the same time to push it anteriorly against the gastric shield.

Barrois ('89–'90, p. 356) believed that the style turned on its long axis while being pushed forward into the stomach. List ('02, p. 277), ignoring this statement of Barrois, expressed the opinion that the function of the large cilia of the style sac was to set the style in rotation, while pushing it forward into the stomach. Hoffmann ('14, p. 533) states that the style of Tagelus is rotated by the cilia of the style sac. In the text of none of these authors is there any statement indicating that the rotation of the style was actually observed. Their conclusions were based merely on the presence of the co-axial layers of which the style is built up.

The secretion and formation of the crystalline style

Regarding the point of origin of the crystalline style there is the same lack of unanimity of opinion among the various investigators that characterizes their speculations regarding its function. In general, three structures have been considered as being the seat of its origin: the stomach, the hepatopancreas, and the epithelium of the style sac.

The opinion of Huxley ('53) was mentioned in the historical summary (p. 58). Milne-Edwards ('59, p. 362) argued from the existence of the co-axial layers of the style that it must be the

product of epithelial secretion. Eighteen years later, Sabatier (pp. 28, 29), after an exhaustive study of the histological structure of the epithelium surrounding the style, found that the cells of this region were full of an unusually large number of protoplasmic granules. He considered that these cells therefore formed a secretory epithelium destined to furnish the stomach with a digestive fluid. Barrois ('89, p. 309) was of the same opinion regarding the origin of the style.

Haseloff ('88) and Hazay ('81), whose conclusions are given in the historical summary (p. 60) believed that the style was a mass of nutriment transformed in the stomach by the action of the gastric juice and stored in the style sac as a reserve. As their conclusions have been so ably disposed of by Barrois ('89), we need not consider them further than to say that they were based on false deductions, and have no valid evidence to support them.

Drew ('01, p. 352) says of Nucula:

> The posterior and part of the lateral walls of the stomach are formed by long and slender epithelial cells that stain but lightly. They secrete a mucus-like material that stains deeply, and probably corresponds to the crystalline style. In adults this structure seldom takes the form of a rod, but in embryos a rod is commonly present.

Mitra ('01, p. 601) says, regarding the secretion of the style,

> There are grounds for believing it is secreted by the so-called liver. The chief ground is that there is in the liver an amylolytic ferment exactly like the ferment of the style. The ferment in the liver behaves exactly as the style ferment does. On the other hand, we could hardly detect any amylolytic ferment in the enteric epithelium. . . . . There is also another fact which must be allowed to have some force in this connection. It is that yellow pigment cells from the liver are occasionally seen to form the axial zone of freshly formed styles.

Biederman ('10–'11) argued from the almost universal presence of ciliated cells in the style-bearing region that, as these could not at the same time be secretory cells, the hepatopancreas was probably the organ of secretion.

By far the greatest weight of evidence, historically, however, places the seat of origin of the style in the typhlosoles. In forms where these do not occur the secretory activity is confined to a region of cells lying in the wall of the style sac itself.

List ('02, p. 274) fed finely ground color particles to Mytilus, and observed that these were built up into the structure of the style. He claims frequently to have seen the style in the process of formation, in which the typhlosoles furnished the secretion in large quantities. He concludes (p. 277), "dass der Krystallistiel im Darme entsteht, und dass hauptsächlich die Secrete der seitlichen Epithelwülste (typholsoles) an seinem Aufbau betheiligt sind."

Gutheil ('11), as a result of an extensive study of the alimentary tract of Anodonta, concluded that a ciliated epithelium might also be absorptive and secretory as well. He found cells in the 'Kristallstieldarm' which were loaded with clear vacuoles lying above the nucleus. These structures he is certain were neither artifacts nor products of degeneration, but material destined to become the style. His conclusions are, therefore, in entire accord with those of List.

My own studies of the secretion and formation of the crystalline style were made on the living material and with stained sections. It is evident from these observations that List was right in placing the origin of the style in the typhlosoles. We have already seen (p. 67) that in forms where the intestine and style sac are incompletely separated by the typhlosoles, a short diverticulum occurs at the end of the style sac. This communicates freely with the intestinal groove down which pass the waste materials from the stomach.

In animals taken during the height of feeding activity, and quickly dissected, a thin thread of large mucus globules may be found issuing from the diverticulum. At the same time there may be seen a string of whitish, ropy mucus, which is secreted by the walls of the intestine for a short distance beyond the diverticulum. The two streams unite at the mouth of the diverticulum and pass anteriorly into the style sac.

The cilia here, as we have already seen, beat so as to put into rotation any matter in contact with them. As a result, the round or flattened stream of mucus is twisted on itself as it starts on its way toward the stomach, thus giving rise to the spiral arrangement typical of the smaller, posterior end of the

style (fig. 14). This spiral structure was recognized by Barrois ('89) and illustrated in several figures.

In the stomach minute sand grains, tests of diatoms, and similar waste matter, entangled in mucus, are beaten by the cilia into compact masses. Passing into the intestine, this waste material is carried along in the deepest part of the intestinal groove. During periods of active feeding some food particles, diatoms, unicellular algae, etc., escape from the stomach and pass down the intestine with the sand and dirt. As this escaped food matter is less compact it comes to lie closer to the typhlosoles, and, if the particles be very small, they may be caught by the cilia on the edges of these structures and carried across to the style. Larger particles, such as the larger diatoms, pass downward until they reach the region of the diverticulum at the end of the style sac. Here they are caught by the cilia and carried into the stream of mucus at this point, while the waste materials pass on through the intestine. Thus it happens that this twisted mucous thread in the style sac so often contains food particles in a very fresh condition.

It has been seen (p. 75) that in the typhlosoles there are two types of secreting cells, long goblet cells, and mucous glands. As the spiral mucous thread is passed anteriorly from the diverticulum, in contact with the edges of the typhlosoles on the right side of the style sac, the secretions from these unicellular glands are carried by ciliary action across the faces of the typhlosoles and applied to it. By the action of the thousands of powerful cilia of the style sac these globules of secretion are beaten into a homogeneous mass around the mucous thread, which forms the core.

As this structure moves anteriorly, turning on its axis, successive layers of secretion are added to it, until as it nears the stomach, further additions cease and the fully formed crystalline style passes out of the style sac into the stomach.

The styles of different individuals of the same species exhibit the most diverse modifications, depending on the physiological state of the animal prior to examination. A specimen which has been starved for some time and then put into water contain-

ing abundant food material will ordinarily show a style of very dark color, due to the large central core of food material. On account of the irregularity in the arrival of this escaped food matter at the end of the caecum, this inner core may vary considerably in thickness throughout its extent (fig. 14). On the other hand, when an animal has had abundant food for a long period and has not been actively feeding just prior to examination, the style may be practically free from all food material and may possess a very thin core of whitish mucus.

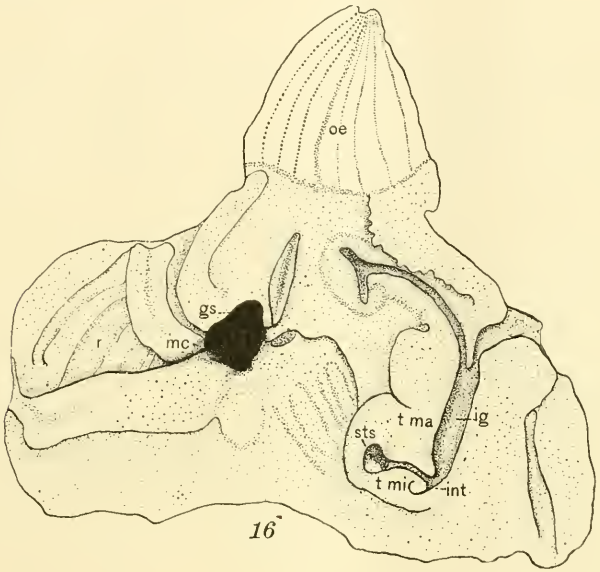
Considerable variation in the length of the secretory portion of the typhlosoles occurs in different species. In some this region, instead of occupying most of the extent of the typhlosole, is quite restricted. As a result, the superficial layers of the style are applied much as a thick tape might be wound around a stick. Such a condition may occur in Mactra where, according to Kellogg ('92, p. 402), the outer lamellae of the style may actually be unwound from the core.

I have found a somewhat similar mode of accretion when feeding Modiolus a fine suspension of carmine grains. The coarse grains of color passed down the intestine to the diverticulum where they were built into the central core of the style. The finer grains, however, were carried across the faces of the typhlosoles at one point and applied to the surface of the style. As a result, this organ when removed much resembled a barber's pole, with the spiral band of bright red carmine grains wound around it.

One interesting anomaly may be described here as shedding some light on the manner in which the superficial layers of the style are laid down. A style of Lampsilis anodontoides was found to which an air bubble had become attached at some distance from the end of the sac. As the style was rolled round and round this bubble was flattened, and over it was deposited a thin layer of clear secretion cementing the bubble securely in place (fig. 17).

The description of the formation of the style as given above applies to all forms in which the style sac is incompletely separated from the intestine by the typhlosoles. In the more primi-

tive forms, where the style lies in the intestine itself and where there are no typhlosoles, the secretory activity seems to be located in the two ridges of cells which form a slight constriction between the style-bearing and the waste-carrying portions of the intestine. The styles of such species exhibit the same inner core and superficial layers found in the styles of Anodonta, Modiolus, etc.



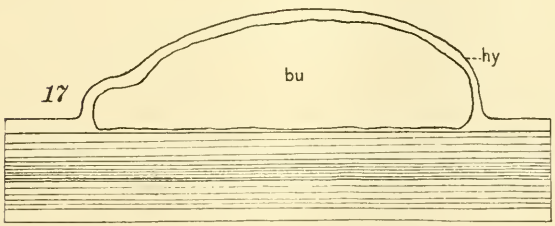


Fig. 16 Anodonta grandis; internal surface of stomach wall, dissected from right side, and spread out flat; *gs*, gastric shield; *ig*, intestinal groove; *int*, intestine; *mc*, mouth of dorsal caecum shown in fig. 4; *oe*, oesophagus; *r*, one of transverse ciliated ridges lining the caecum and stomach wall adjacent to it; *sts*, opening of style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

Fig. 17 Lampsilis anodontoides; optical section of crystalline style with attached air bubble. *bu*, bubble; *hy*, hyaline layers.

In those lamellibranchs where the style lies in a separate caecum, distinct from the intestine, there are marked differences from the type we have been considering. Since there is no connection between the style caecum and intestine, there is no means by which food particles could be incorporated into the structure of the style. Investigators who have examined the styles from this type of mollusc state that food particles are universally absent from them. In Pholas, for instance, following Barrois ('89), the style has a central core of bubbly mucous, entirely free from foreign particles. I find the same is true of Martesia.

Mitra ('01) noted the inner core of food matter in the style of Anodonta, but thought that it was due to some imperfection in the method of storing the ferment in the style sac. The absence of such an inner core in the styles of Pholas was held to be the result of a superior, more differentiated mechanism for storing the ferment and for passing the food material through the alimentary canal.

A further difference in this species lies in the fact that the style is comparatively short and thick and that the smaller and not the larger end projects into the stomach. I have found this condition in Martesia, where the style is of relatively enormous proportions, comprising as it does a large part of the visceral mass (fig. 8). In both of these forms the style resembles roughly a policeman's club in shape.

The relatively large style in Teredo and other boring molluscs was noted by Deshayes (48) and Quatrefages ('49).

I have been unable to find any reference in the literature as to the exact location of the secretory activity in forms possessing a separate style caecum. We have seen that Mitra's supposition that the hepatopancreas is the source of this secretion is entirely opposed to the facts as determined for the species thus far considered. That this supposition is equally invalid in the type under consideration must be evident from the anatomical relations of the hepatopancreas to the style caecum, as found in Martesia (fig. 8). To my knowledge no channel exists through which a secretion from the hepatopancreas could be applied to the surface of the style, even if the effective stroke of the cilia of this

portion of the alimentary canal were such as to carry a secretion to this point. Furthermore, I find at one side of the caecum itself a group of cells which form a structure very similar to the secreting epithelium of other forms. It may be concluded, therefore, that where the style exists in a separate caecum it is likewise the product of an epithelial secretion.

Embryology of the style and style-bearing organs

Concerning the ontogenetic development of the crystalline style the literature is singularly silent. Reference to it is found in the work of Drew ('01) in the passage quoted above (p. 81) and also in the following words. Speaking of the alimentary tract of the veliger larva of Nucula, he says (p. 349):

> The cells of the epithelial walls of the stomach are of two kinds. Those at the anterior end of the stomach carry comparatively few cilia and those at the posterior end carry many cilia. At this stage some of the cells on the dorsal side of the stomach, near its anterior end, begin to secrete a mucus-like material that extends posteriorly in the lumen of the stomach as a small rod that probably represents the crystaline style. Later the posterior portion of the whole dorsal division of the stomach . . . . is given over to secreting this material, but a definite rod may not be present.

In the same year Meisenheimer described the development of the crystalline style in Dreissensia, a form with a distinct style caecum. According to this investigator (p. 93), the anlage of the style caecum lies in the posterior wall of the stomach, to the right of the opening of the intestine. This region is characterized by the presence of very long bristle-like cilia (fig. 5). The epithelium of the stomach wall at this point evaginates to form a cone-like depression, the cells of which secrete a substance which is formed into a thick rod extending into the stomach (fig. 6). This blind sac continues to grow posteriorly until it finally forms a long caecum on the right side of the body. It opens into the stomach to the right of the intestinal orifice. The food of the larva of this species consists of plankton, but in consequence of the presence of hard particles in the stomach, incidentally acquired during feeding, Meisenheimer favors Bar-

rois' theory of the protective action of the dissolved style substance.

My own investigations of the embryological development of the crystalline style were carried on during three summers on the New Jersey coast, while engaged in a study of the food and feeding habits of the veliger larvae of Ostrea and Venus.

In the prodissoconch larva of Ostrea I find in the epithelium of the stomach the same two regions, differing in the cilia they bear, as were described by Drew for Nucula. Most of the stomach wall, like that of the oesophagus, is covered with short, even cilia which beat at a uniform rate. Just anterior to the middle of the stomach, and situated on opposite walls, are two groups of long cilia which beat much more strongly than the others. They coincide closely in position with those long cilia which have already been described in the adult (p. 77) which are situated on either side of the gastric shield and keep the alimentary matter in circular motion. As was pointed out by Meisenheimer ('01, p. 94), the food material in the stomach of the larva is in constant rotation under the action of the cilia. In Venus and Ostrea this motion is due largely to the action of the two groups of large cilia; it is always in a clockwise direction when viewing the animal from the anterior end. These cilia, both in position and in the direction of their effective stroke, correspond closely, therefore, to those found in the adult.

When these veligers are placed in water containing carmine grains in suspension, they feed upon it greedily and soon fill the entire alimentary canal. As the color grains enter the stomach they are caught by the large cilia and whipped into the mass of particles and mucus which is whirling in the cavity of this organ. This circular motion is not continuous, for the larvae exhibit spasmodic contractions of the velum and foot, as a result of which the stomach is compressed and the whirling motion ceases for a moment, to be resumed upon the relaxation of the surrounding organs. During the rotation of the alimentary mass the particles of carmine or food matter move slowly posteriorly into the opening of the intestine, where they are caught and carried downwards. At this stage there is no indication of the beginning of a separate intestine and style sac.

Such a condition is present in the oyster larva for the first week or ten days. As the free-swimming stage reaches its close, and the oyster larva attaches itself to some foundation, the mucous roll of the stomach is represented by a short rod extending backwards into the lumen of the intestine. Meanwhile the intestine becomes constricted lengthwise to form two tubes. In an oyster $\frac{4}{5}$ mm. in diameter the organs are well differentiated and a distinct style is present. In a 9-mm. individual this differentiation is apparently complete. The style is comparatively short and thick and is as clear as crystal. It shows the same twisted inner core as is found in the adult. The gastric shield is likewise well developed at this stage. In a 9-mm. oyster it measures approximately 0.7 x 0.5 mm. The style of a 7-mm. oyster measures about 1.2 mm. long and 0.3 mm. in diameter at its widest extent.

At this time the young oyster has all of the organs for food-getting of the adult and eats the same food. We may suppose, therefore, that the style now serves it in the same manner as it does the adult.

3. NATURE

Description of the style

The length of the style is described by Mitra ('01) as fully three-fourths that of the animal. Gutheil ('11) gives a more conservative estimate, the style from an Anodonta 13 to 14 cm., measuring from 6 to 7.5 cm. Biederman ('10,–'11) gives the length of the style of an adult Anodonta as 7 to 8 cm. My own observations agree closely with those of the last two investigators, though the length of the style varies greatly with the species and the feeding activity of the animal.

Figure 14 shows the appearance of the style as taken from its sac, with a mass of food material attached to the anterior end. This figure gives an optical section through a style containing a thick core of food material, showing the spiral arrangement of this inner core and the layers of hyaline substance which have been secreted upon it.

In color the crystalline style varies from a clear, transparent structure, like a glass rod, to a deep brown, opaque rod, the color of chocolate. Aside from the presence of an inner core of food material, which may give more or less color to the styles of all species not having a separate caecum, the cortical layers of hyaline matter themselves may contain a diffuse yellow substance. In Anodonta and the adult Ostrea I have invariably found some trace of this color present, even in the clearest styles. In Lampsilis, Modiolus, Martesia, and the very young oyster, the cortical layers are usually as clear as crystal.

The composition of the style

Investigation of the composition of the style by the earlier workers—von Poli (1791), Hazay ('81), and Haseloff ('88)—revealed the following facts: gelatinous in consistency, the style dissolves readily in water, more rapidly in salt solution. It is coagulated by boiling water, alcohol, and other protein precipitants. It gives the delicate KCN reaction for albumen, and a violet color with the biuret test.

The most extensive investigation of the structure and chemcal composition of the crystalline style which has yet appeared is found in the work of Barrois ('89). A chemical analysis of the style by Lambling is included in this paper. The conclusions of Barrois are based not only upon many different forms, but what is of equal importance, upon a large number of individuals of each species. In following the color variations of the style this investigator opened over 6,000 individuals of Cardium alone. Since this work has been given but scant attention by most subsequent writers, the results are given below in some detail.

The style was found to vary in size with the individual and with the relative proportions of the sac or caecum containing it. The longest style described by Barrois was 7 to 8 cm. in Anodonta anatina. As found in the marine species the style was usualy of a pale yellow color, while in the Unionidae it was transparent and colorless. Great variation was found in the

color in Cardium during the year, being a deep orange in April, when it was most highly colored.

Much variation in the consistency of the style was observed in the different species, this difference being correlated with the rapidity with which the structure underwent dissolution. As found in the Unionidae, Mytilus, and Ostrea, it was soft and gelatinous, becoming much firmer in Cardium, Solen, Donax, and Pholas. The brown inner core of food material was held by Barrois to be abnormal. He noted that the style of Pholas never contained such a structure. The coaxial layers in the style of this species were thicker near the center and much thinner toward the periphery, grading from 9 to 35 microns, with an average of about 14 microns. The style of an adult Pholas crispata showed from one hundred to one hundred and ten successive layers.

In the cortical layers of the style Barrois found small crystalline bodies which were insoluble in water and resistant to acetic acid. These were undoubtedly the same structures described by Vulpian ('67) as crystals of calcium oxalate. Since the latter investigator also found uric-acid crystals in some styles, he concluded that the crystalline style must have some connection with the excretory function. Similar crystals were described by Hazay ('81), as semitransparent, ruby-colored rhombohedrons.

I have found such crystals in the styles of Anodonta and Ostrea. They are usually red or jet black, the former resembling in color the lipochrome pigment cells in the integument of the crayfish. These crystalline bodies are not normally present in the style, but appear after partial desiccation of the style substance.

A fairly complete chemical analysis, made by Lambling, is given by Barrois, '89. The style used was that of Cardium edulis, which in an adult specimen is about 26 mm. long and weighs approximately 0.026 g.

His analysis of fifty styles gave the following results:

	grams
Weight of fresh styles	1.3225
Weight dried at 120°C	0.1705
Insoluble mineral matter	0.0025
Soluble mineral matter	0.0090

Expressed in percentage weight of the fresh styles gives:

		per cent
Water		87.11
Solid matter	organic	12.03
	inorganic	0.86

The albuminous nature of the style substance was shown by the following reactions. The styles were washed rapidly by decantation and dissolved in distilled water. The solution was neutral. Conc. HCl, Millon's reagent, biuret and Adamkiewicz tests were all strongly positive. Boiled with a 2 per cent solution of H_2SO_4, neutralized and precipitated with alcohol, taken to dryness, and dissolved in water, the style substance gave a strong reduction with Fehling's solution, similar to that given by mucin and chondrin, but might have been due to the saccharification of a hydrocarbon. All attempts to find such a hydrocarbon were futile.

Maillard and Vles ('07) found a similar reduction when a solution of styles, containing one part to a hundred of sodium fluoride was tested with Fehling's solution. This reduction, though feeble, was constant. That it was not due to the presence of a sugar was shown by a negative reaction with phenylhydrazine.

Barrois tested this reducing action of the style and found it to be equal in reducing power to 0.059 gm. of glucose, per gm. of dry protein matter present. Whatever this reducing substance might have been, it was not optically active.

Passing a current of CO_2 through a solution of styles, and also saturation with $MgSO_4$ after the method of Hammarsten for blood serum, showed that practically all of the proteid matter of the style exists as a globulin.

In conclusion; Barrois points out as most important the action of hot weak acids on the style, a reaction similar to that shown by the albuminoid matter of mucin and chondrin. These, he holds, are protein constituents, which in animals of higher organization, at least, appear to us as albuminoids which are more or less non-catabolized (dégradés), that is, no longer fill a biological rôle in the phenomena of general nutrition, and hence

cannot serve as a reserve of nutriment. List ('02) agreed with Barrois that the style is similar to mucin and chondrin.

Mitra ('01), ignorant of the work of Barrois, repeated most of the chemical analyses of Lambling, using the styles of Anodonta. His results in the main are of a confirmatory nature. He states that the style contains no cellular elements, but is composed of a colloid substance. Its solubility, according to him, is due to the presence of a minute quantity of salts. His tests for protein were similar to those of Lambling and revealed the same facts. His chemical analysis (p. 599) showed about 88 per cent water, about 12 per cent of protein (globulin), and about 1 per cent of salts, which is a close approximation to the results of Lambling.

The credit for first determining the presence of enzymes in the crystalline style has been given to Mitra ('01) by nearly all subsequent investigators. However, as van Rynberk ('08) points out, the credit for this discovery belongs to Coupin ('00), his work appearing a year before that of Mitra.

In a short note Coupin gives a summary of his results showing that the style contains no sugar or fat, but only an albuminoid substance. He concludes:

> Le stylet Crystallin des acéphales est un suc digestif, un espèce de *comprime de diastase*, contenant beaucoup d'amylase, et peu de sucrase, melees avec une substance muqueuse, destinee sans aucun doute a empêcher la trop rapide dilution du stylet dans l'eau de mer contient l'estomac, et peutêtre aussi à agglutiner les particlles solides qui nagent dans celli-ci.

He found, further, that the enzymes of the style were without action upon egg albumen.

Mitra showed that an aqueous solution of styles caused a rapid conversion of starch to sugar, with an intermediate product in the nature of dextrin. Its activity toward glycogen was found to be similar to that of ptyalin. No action could be demonstrated on egg albumen, fibrin, or muscle fibers.

An extract of the hepatopancreas showed the same activity as the style, while the extract of the mid intestine revealed hardly a trace of this digestive action.

The alcohol precipitate from an aqueous solution of styles was kept under alcohol for several months, and finding that the ferment power of this precipitate became increasingly less as it became more insoluble in water, Mitra concluded that the enzyme and protein of the style were identical.

Von Fürth, reviewing part of the literature, believed that it was more reasonable to suppose, with Coupin, that from the colloidal nature of the style it was really a mixture of enzyme and albuminoid material, rather than a solid mass of enzyme.

Van Rynberk ('08) repeated the experiments of Coupin and Mitra, and extended them to cover a comparison of the digestive activity of the style and the hepatopancreas on fats, proteins, and carbohydrates. This author found no action upon fats or proteids, nor upon cellulose, though starch was quickly digested, and raw sugar inverted by the enzymes present, in both the style and hepatopancreas.

My own investigations, as far as carried out, confirm those of Coupin and van Rynberk, and differ from those of Mitra only in so far as he regarded the style as exclusively a mass of enzyme. When digestion is proceeding slowly in Anodonta, as during the winter, the stomach contains a large amount of brown, ropy fluid, resulting partly from the dissolution of the style, and partly from secretions from the hepatopancreas. About 0.5 cc. of this liquid was drawn off and mixed with an equal volume of a 0.5 per cent starch solution, and the mixture placed at 32°C. In an hour the starch was completely digested. An extract of the hepatopancreas showed a similar activity, though the action was not nearly as rapid.

Mitra extracted the mid intestine and found a very faint amylolytic action of the filtrate. Van Rynberk repeated the work, but failed to confirm his result.

Since the secreting glands of the typhlosoles are all unicellular, it is evident that little enzyme could be stored in them. The following experiment was designed to avoid this possible source of error. The typhlosoles, together with the wall of the style sac, were carefully removed from an adult Anodonta. That part near the hepatopancreas was discarded, and the rest, after

rapid washing in distilled water, was placed in a vial with a small quantity of the mollusc's blood. A few drops of the glycerine and twenty drops of a 0.5 per cent starch solution were added and the vial placed at 32°C. At the same time a control containing blood and starch was also put in the incubator. The object of putting the tissue in the blood was to give the cells a chance to secrete a small amount of enzyme while bathed in fluid approximating that of the body. After twenty-two hours the control still showed the presence of starch, while in the vial containing the tissue all of the starch had been converted. It is evident, therefore, that the enzyme found in the style is secreted by the typhlosoles, and not by the hepatopancreas as was held by Mitra.

Apparently the molluscs which I have studied have no extracellular lipase. Van Rynberk ('08) could demonstrate no action upon fat, and I have found in the alimentary mass in the stomach of Anodonta great numbers of fat globules, as shown by their staining reaction with Sudan III. It is probable, furthermore, from the researches of Gutheil ('11) and the recent work of Churchill ('16), that fat is absorbed as such by the cells, and by phagocytes, there to undergo decomposition into fatty acid and glycerine.

The only addition which I have made to the chemical analysis of Lambling is the determination of the total and water-soluble nitrogen in the style.

Ten styles of Lampsilis were washed rapidly in distilled water, drained on filter paper, and weighed. Two cc. of distilled water were added and, when the styles had dissolved, 2 cc. of a mixture of tannic and hydrochloric acids were added to precipitate the protein matter. The precipitate was filtered off through a small, dry, nitrogen-free filter paper, and the filtrate analyzed for the water soluble nitrogen. The residue on the filter was washed free of all traces of the filtrate, and, with the filter paper, was digested in boiling conc. H_2SO_4, and the amount of nitrogen determined by the micro-Kjeldahl method. The results of this experiment follow:

	gram
Ten styles, fresh	0.16
Nitrogen, per g. of fresh style	
Water soluble	0.0011
Precipitable	0.0061
Total nitrogen	0.0072

In criticism of Mitra's conception of the style as a mass of pure enzyme, it may be urged that the evidence which he advances is inconclusive. The mere fact that the style substance, kept under alcohol, lost in ferment power, proves nothing, for it is well known that enzymes in general lose their activity on long standing under such conditions.

Furthermore, examination of the substance of a freshly formed style with the dark stage condenser shows it to be composed of small globules of fairly uniform-size, interspersed with many very minute globules or particles, in the nature of colloid particles. It is probable that we have to do with an adsorption phenomenon, the enzyme being borne on the surface of the globules of albuminoid substance.

It may be concluded, therefore, following Coupin ('00), that the crystalline style is a structure of colloid nature, resembling mucin, and containing an enzyme, or enzymes, of strong amylolytic power.

Nature of the gastric shield

Finally, a few observations are necessary regarding the nature of the gastric shield. Subjected to the biuret test, it rapidly disintegrates under the action of the strong caustic. On the addition of the copper-sulphate solution a purple color forms on the surface, grading into a pink toward the interior. This would indicate the presence of such substances as proteoses and peptones. From the zonation of the colors it is probable that this reaction is due to products of digestion absorbed by the shield. From its consistency and action toward the common reagents it is probably in the nature of chondrin.

The shield is very resistant to the action of the digestive juices of the stomach. I have invariably found it present in all living specimens that I have examined. If kept in a test-tube with a

small amount of digestive juice, it slowly softens and, after several days to a week, disintegrates. It is therefore, a structure, which, unlike the style, is not renewed at the commencement of feeding activity.

The Spirochaetes of the crystalline style

As is well known, the crystalline style in many species of lamellibranchs harbors a large spirochaete, which has been put by Gross ('10) in a separate group, the Cristispira. My own observations have been on Cristispira balbiani Certes, from the oyster, and C. anodontae Keysselitz, from Anodonta grandis, one of the largest spirochaetes known. Why they occur in the style and whether or not they are harmful to the host are questions which cannot be considered in the present work. These organisms are mentioned here only because they throw some additional light on the consistency of the style substance.

Examination of a fresh style of Ostrea or of Anodonta shows the spirochaetes in great numbers, moving back and forth with their characteristic corkscrew motion. The majority of these Protozoa occur at or near the surface of the style, but apparently they have no difficulty in passing inward to the core and back again through the substance of the cortical layers. Fixed sections show them in all parts from the core outward

In Anodonta, at least, these organisms are usually found in the greatest numbers in the bubbly mucous in the end diverticulum of the style sac, becoming much fewer toward the stomach. It is probable that their presence near the anterior end of the style and in the stomach is due to the continual forward movement of the former, and not to choice on the part of the spirochaetes.

A striking peculiarity in their distribution lies in the fact that the styles of some species appear to be wholly free from them. Such is the case in the styles of Lampsilis luteolus and L. anodontoides that I have examined, though in one instance twelve spirochaetes were discovered in a style of L. anodontoides.

Also, I have never found any spirochaetes in the styles of Modiolus, though oysters attached to them are heavily infected.

Finally, the spirochaetes, unlike most protozoans, are able to resist the action of the digestive ferments of the alimentary canal, and are apparently unacted on by the juices in the stomach.

4. FUNCTION

The conclusions of former investigators regarding the function of the crystalline style have been considered in the historical summary (p. 57). The inadmissibility of most of these must be evident at once, and they will be dismissed without further comment. A critical examination of the others is reserved until after the presentation of my own conclusions and the evidence upon which they are based.

Realizing that the misconceptions of many former workers have been due largely to abnormal and unfavorable conditions surrounding the mollusc under observation, my own endeavors have been to study the style in its physiological relationships while the animal was still in or near its natural environment.

In this I have been greatly aided by the opportunity afforded by the floating laboratory for oyster research of the New Jersey Agricultural Experiment Station. As this laboratory is stationed over the natural beds of the oyster, with clams and mussels within a few feet, it was possible to remove a bivalve from its natural environment and within two or three minutes to have it opened and under observation in the laboratory. Too much emphasis cannot be laid on the necessity for study of this nature in determining the physiological significance of the style.

The first investigations covered a determination of the factors in the normal environment which bring about the secretion or dissolution of the style.

The ribbed mussel, Modiolus, occurs most commonly in bunches along the banks of tidal creeks and estuaries. Natural oysters also may be attached to these clusters. From their position on the bank, the molluscs are necessarily exposed from three, to as much as six or eight hours between successive high tides, during which time, of course, no feeding takes place.

Upon examining mussels which had thus been exposed for about six hours, it was found that the style was invariably present. In many instances, however, it was quite soft and had begun to undergo dissolution.

The oysters attached to these mussels, provided they had been exposed for an hour or more, failed to show any style present. What, then, is the relation of the style to the feeding activities of the oyster? As is well known, oysters occur naturally in reefs, or barriers, built up through successive generations attaching themselves to those already present. Since the oyster is able to exist for some time out of water, the upper limit of the reef comes to lie between the mean low- and high-water levels. Consequently, large numbers of individuals near the top are exposed for several hours at each ebb tide.

Toward the close of ebb tide I went out on such a reef and opened oysters at the rate of one a minute for two and a half hours, a period extending over the close of the ebb and the early flood of the tide. Began at 9.05 a.m., at which time the oysters around me were all or partly submerged. A style was present in all that were opened.

By 9.45 a.m. most of the reef about me was exposed, and the oysters opened for the next hour were devoid of styles. Shortly after 10.00 a.m. the tide began to return, and when it had risen sufficiently to cover the oysters they opened and began siphoning vigorously. By 11.00 a.m. the reef was practically covered, and from then on till 11.35 a.m., when the experiment was concluded, a style was present in every oyster opened. All the bivalves taken during the entire period came from a spot less than 20 feet square and nearly level, hence all were subjected to practically the same period of exposure.

Subsequent laboratory experiments confirmed these results and revealed the fact that the style of the oyster may be dissolved within an hour, often in much less time. Furthermore, a new style may be built up within fifteen minutes from the time the animal begins active feeding.

There is, however, a great variation in the length of time required for these processes, depending on the previous feeding

activity of the mollusc, as well as its general physiological condition. External conditions, principally temperature, also cause considerable variation in the time required.[3]

Upon dissolution the style forms a brown viscous fluid, most of which remains in the style sac until the animal again begins active feeding, when it is carried forward into the stomach by the active beating of the cilia.

From the above experiments it may be concluded that the crystalline style is a structure intimately connected with the feeding activities of the mollusc, and which, in oysters exposed at low tide, is completely dissolved and renewed again at least twice every twenty-four hours.

The style of Modiolus is much more resistant and ordinarily remains intact during the normal periods of inactivity. If mussels are kept in the laboratory without food, the style begins to disappear after about twelve hours, and is usually completely dissolved after twenty-four hours of inactivity, though here again there is great variation between individuals.

The fresh-water genera, Anodonta and Lampsilis, exhibit a condition similar to that of Modiolus. Absence of food or inactivity of the animal due to cold or adverse conditions brings about a gradual dissolution of the style, occupying from a few hours to several days.

The disappearance of the style in forms which had long been out of water was noted by Meckel ('29) and by a number of other workers. Mitra ('01) believed this disappearance and subsequent renewal to be a periodic function. As Allen ('14, p. 136) has justly pointed out, this periodicity was due to the periodic emptying and filling of the aquarium in which the animals were kept. The latter investigator also gives the correct interpretation of the presence or absence of the style, and showed that the addition of food to the water containing starved mussels soon caused its partial regeneration.

We have already seen (p. 80) that the cilia of the style sac put the style into rotation while pushing it forward into the

[3] The rapid dissolution of the style of the oyster was noted by Möbius ('83) and later by Barrois ('89). Garner ('41) even went so far as to hold that with but few exceptions the style was lacking in all of the Monomyaria.

stomach. Though surmised by several former workers from the co-axial layers of which the style is composed, I am unable to find any statement in the literature indicating that this rotation has actually been observed.

After nearly two years of experimenting, in which many individuals, both marine and fresh-water, were opened, I was able finally to cut through the stomach wall and expose the head of the style in such a way that the animal did not contract unduly, with consequent displacement of the alimentary canal.[4]

As a result the style is not put under tension, but is free to rotate in the style sac, in spite of the fact that it forms a U-shaped bend in following the course of the intestine. To my surprise, I found this rotation to be quite rapid. In Anodonta kept at a constant temperature of 11.5°C. prior to, and during the experiment, the maximum number of revolutions per minute was eleven. In Modiolus, at 25°C., the maximum number per minute was thirteen. The direction of rotation in both species is clockwise when viewing the animal from the anterior end. (Compare this with the direction of rotation of the food particles in the stomach of the veliger larvae of Ostrea and Venus, p. 88.)

The food material in the stomach, entangled in mucus, becomes wound about the head of the style, and whirled around in the lumen just posterior to the gastric shield. So strong is the tractive force of the rotating style that strings of mucus from any part of the body, if led to the stomach cavity, are at once drawn in and wound up in the food mass.

While rotating the style in this manner, the cilia of the style sac push it anteriorly against the gastric shield, with force enough to cause the style to bow out when the stomach walls are drawn apart. In Modiolus, at 25°C., this forward move-

[4] The technique employed is as follows: An individual which has been siphoning vigorously is removed from the water, and the right valve removed by passing a sharp, thin scalpel between the shell and the adductor muscles, leaving the latter intact. The mantle and gills are then laid back, and a small incision is made in the body wall over the dorsolateral region of the stomach. At this point the stomach wall comes very close to the surface in those species I have studied. By means of fine hooks, the cut edges of the stomach are pulled apart to expose the head of the style where it bears against the gastric shield.

ment, when the end of the style is unopposed, is approximately 1 cm. per minute.

We have already seen (p. 77) that the cilia on the stomach walls in proximity to the gastric shield beat so as to rotate in a clockwise direction any matter in contact with them. They thus aid the movement of the style in keeping the food material in quite rapid rotation.

The tip of the style, enclosed in a mass of alimentary matter is constantly dissolving away as it is pushed up against the gastric shield. This dissolution is, however, confined to the cortical layers, the central core of bubbly mucus remains undissolved and is wound up with the rest of the mass around the head of the style.

The causes for the dissolution of the style consist, first, in the action of the juices of the stomach; second, the wearing against the point of the shield, and, third, the tendency of the style to dissolve spontaneously within a certain time after formation.

Figure 12 shows the oesophagus, stomach and style-bearing organs of Modiolus. In two favorable specimens I was able to cut through the stomach wall and observe the movement of the style with the surrounding alimentary mass, while strings of food and mucus from the gills and palps were passing up the oesophagus. It was therefore possible to observe the action of the various parts while they were functioning in an approximately normal manner.

The food particles, with large numbers of sand grains, entangled in mucus, pass up the oesophagus in a single string and, bridging the groove at the entrance to the stomach, are carried to the base of the gastric shield. Here they are caught in the revolving alimentary mass and wound around it. The mass, in turning, comes in contact with the spiral cavity described on page 78 and shown in figure 12. As this mixture of mucus, food particles, and foreign matter sweeps across the highly ciliated grooves of this structure, sand grains, together with much of the newly arrived matter from the oesophagus, are caught out of the mass and carried down the tract and into the right side of the caecum (p. 78 and fig. 15).

Here the mechanism described above (p. 78) comes into play, and by means of this most of the sand grains are separated from the food particles and are carried into the intestinal groove, while the latter pass out of the left side of the caecum. From here the ciliary currents carry the food across the ventral wall of the stomach to the right side of the gastric shield, where it again enters the revolving alimentary mass (fig. 12).

In the two mussels which I had under observation a steady stream of sand and food particles was entering the right side of the caecum, while a stream of practically pure food particles was issuing from the left side. At the same time pellets composed almost exclusively of sand grains were passing along the intestinal groove and into the intestine. From time to time small portions of the three streams just described were removed and examined microscopically, and revealed the fact that practically complete separation of food and sand was taking place in the caecum.

By means of this mechanism the ingested material in the stomach is gradually sorted over and purified, until, when feeding has ceased, the stomach contains a mass of pure food material.

In all cases where I have had the revolving style under observation the motion is not continuous, but is interrupted by periods of inactivity, preceded by a gradual slowing down of the style. During these periods of cessation the cilia of the style sac themselves become greatly reduced in activity. After a period of rest the cilia again begin to beat actively, followed by a resumption of motion of the style. What the stimuli may be that cause the cessation and resumption of motion on the part of the style I have been unable thus far to determine. The presence or absence of food in the stomach itself seems to be of no consequence so far as this activity is concerned. It is possible that the large bristle-like cilia of the style sac may be under the control of the nervous system and respond to certain internal reflex stimuli called forth by the stage of digestion of the animal.

We have seen that the ribbed mussel lives under conditions such that its feeding periods are usually restricted to a few hours. As a result, it makes the most of every opportunity, and if one examines a cluster of these molluscs after the rising tide has just covered them, the surface of the water will be seen boiling like a miniature spring, due to the very active siphoning.

When removed to a dish containing sea-water with much fine sand in suspension, the water is soon completely filtered by the activity of these molluscs. Comparatively little discrimination is made by the palps and gills, save against the larger particles; everything else is taken into the stomach and sorted there. Within about an hour after placing a mussel in roily water it will begin to void faeces in a continuous ribbon, composed almost wholly of sand grains held together by thick mucus.

In a recent paper on the ciliary mechanism of lamellibranchs, Kellogg ('15, p. 660) describes the peculiar 'sand-eating' genus Macoma. He states that the entire digestive tract of this form is filled with debris. Since the backwardly directed currents of the palps and mantle are as well developed in this as in other species, Kellogg is unable to account for the ingestion of such large quantities of silt. In view of the fact that Modiolus, though possessing well-marked outgoing currents, ingests much sand along with its food, it is probable that Macoma will show upon investigation some similar means of sorting out the food materials from foreign matter in the alimentary canal. A further point of similarity between these two species lies in Kellogg's observation of the extraordinary power of the cilia in this form. My own observations on Modiolus indicate that the outgoing tracts are for the purpose of removing the larger particles which do not enter the mouth.

As we have seen above (p. 71), Anodonta possesses a diverticulum which somewhat resembles the 'sand-sorting' caecum of Modiolus, though less well defined. Space will not permit of a description of analogous structures in the various forms studied. Suffice it to say that Lampsilis, Ostrea, and Venus all show some modification of the stomach wall in connection with the intestinal groove and extension of the major typhlosole, by means of

which a certain amount of selection and separation of the material in the stomach does take place.

In artificial feeding experiments I have found that the selective action of the palps of Ostrea is very great as compared with that of Modiolus, even the most finely divided carmine grains being rejected. Coincident with the increase of selective power on the surface of the body there is a corresponding lack of specialization of the wall of the stomach for sorting over the alimentary matter.

Summing up what has been said of the origin of the style in the developing embryo (p. 88), together with the above description of the process of feeding in the adult Modiolus, it is evident that one of the primary and important functions of the crystalline style throughout the life of the lamellibranch is to aid in keeping the alimentary mass in the stomach in motion.[5] This movement is necessary, not only as a means of sorting out foreign matter in the stomach, but also as a substitute for muscular peristalsis in keeping the food in motion.

Since the head of the style with sand grains and other rough objects attached to it bears against the stomach wall, there is necessarily developed at this point a resistant structure to prevent injury to the epithelium. The gastric shield therefore arose as such an organ of protection, as was first believed by Barrois, who compared it to the 'Trichter' of insects and some other arthropods.

Barrois, followed by Pelseneer ('91) and others, believed that the function of the style, when dissolved, consisted in surrounding sharp particles and thus preventing injury to the epithelium. Against this interpretation I would urge the following objections: First, the style, allowed to dissolve of itself without the addition of any fluid, forms, with the exception of the inner core, a liquid of such a thin consistency as to be of little or no possible value as a protective covering; second, the cells of the alimentary tract are covered with a heavy carpet of large cilia, so that no sharp object would be likely to come in contact with

[5] Milne-Edwards ('59) was the first to advance this theory, but without the least evidence in its support.

the surface of the cells; third, the streams of food and foreign matter entering from the oesophagus are largely composed of heavy mucus secreted by the gills and palps, and this is in sufficient quantity and of a consistency capable of acting as a protective covering if such were needed; fourth, an examination of the pellets of sand passed down the intestinal groove of Modiolus shows them to be composed of sand grains compactly held together by a small amount of thick mucus, such as that secreted by the body surface or oesophagus.

A second function of the crystalline style, though probably a minor one, has been referred to above (p. 83) in describing the formation of the style, where it was shown how undigested food materials, passed down the intestine along with the waste matter, are caught and incorporated into the inner core of the style.

During the winter I have found individuals in which practically the entire style was composed of the brown particles which form so large a part of the alimentary mass of the stomach. The crystalline style thus serves incidentally, except in those forms in which it lodges in a separate caecum, as a means of restoring to the stomach nutriment which might otherwise be lost.

One is greatly tempted at this point to consider the endostyle of Amphioxus, in so far as it acts in the transfer of food, as possibly an analogous structure. However, as I have been unable to examine living specimens of this animal, this similarity is merely suggested here.

Finally, we have seen that the crystalline style of the lamellibranchs contains a very active amylolytic ferment, as was first held by Coupin ('00), followed by Mitra ('01), van Rynberk ('08), and others. From the evidence presented by these workers, together with the facts shown in this paper, it is probable that the enzyme is held in a viscous matrix of a mucin-like substance which gives the reactions of a globulin.

Since the food of the majority of lamellibranchs consists mainly of unicellular plants, and since these animals have no salivary secretion, an abundant supply of starch-splitting enzymes in the alimentary canal is a necessity.

The presence of a style of great dimensions has been noted by several workers in Teredo and other boring molluscs, and I have found the same in Martesia. The relatively great development of the style in these forms is probably connected with their mode of feeding and living.

Some of these molluscs bore into objects close to the high-tide level, and are hence unable to feed for long periods. All of them communicate with the exterior by means of a comparatively small opening. It is probable that their periods of feeding are further disturbed by deposits of mud over these openings, so that it is necessary to use to the best possible advantage every opportunity for feeding. Such being the case, the presence of a large amount of enzyme, ready for immediate use, could not but be of great advantage to these molluscs.

5. SUMMARY AND CONCLUSIONS

In the present paper the attempt has been made to bring under one head the results of all former workers regarding the crystalline style of lamellibranchs and to settle important questions concerning its origin, nature, and function.

A survey of the literature shows that much of the credit given to Mitra ('01) rightfully belongs to Barrois ('89, '90) and Coupin ('00).

Anatomical features of the stomach and intestine of some common lamellibranchs, not previously noticed, are pictured and described.

The mode of secretion and formation of the style has been studied in marine and fresh-water forms. It arises as a thin core of bubbly mucus, upon which are deposited co-axial layers of a gelatinous protein, containing enzymes.

Its embryological development is followed in Ostrea and Dreissensia.

The ciliary mechanism of the stomach, intestine, and style sac has been traced out in some detail. It was found that the molluscs studied possess the ability of separating food from foreign particles in the stomach by ciliary action. This mechan-

ism was found best developed in those species, like Modiolus, in which little discrimination is shown by the gills, palps, and mantle.

The actual rotation of the style was observed, and is here described for the first time. This movement of the style is of great importance in separating the food from foreign particles and in serving as a substitute for peristalsis.

The gastric shield is shown to be an organ for the protection of the gastric mucosa against the abrasive action of the head of the revolving style.

In those species in which the style sac is incompletely separated from the intestine, the style serves as a means of restoring to the stomach undigested food particles which might otherwise be lost.

The conclusions of Coupin ('00), that the style contains strong amylolytic ferments, have been confirmed. These enzymes are held in a stiff gelatinous matrix of a globulin-like substance.

In some of the boring molluscs the style attains a relatively great size, thus representing a large mass of enzyme for immediate use.

The conclusions of Mitra ('01), regarding the style as solely a mass of pure enzyme secreted by the hepatopancreas, and the theory of the protective action of the style substance, held by Barrois, Pelseneer, and others, are hereby rendered untenable.

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THE OLFACTORY ORGANS OF A COLEOPTEROUS LARVA

N. E. McINDOO

From the Bureau of Entomology at Washington, D. C.

THIRTY-THREE FIGURES

INTRODUCTION

The olfactory sense of adult insects is highly developed and perhaps no other animal can smell as well as the honey bee; in fact it seems that this sense has been the chief factor by which the social life of insects has been acquired. Among honey bees the olfactory sense serves not only as a means of distinguishing the three castes and the different individuals in the same colony, but it is also vitally important in scenting and selecting the proper food. During the immature stages of insects, sexual odors, should they be emitted, probably play no rôle as such odors, but substances suitable for food are constantly emitting odors, and since food is more necessary to larval forms of insects than to the adult forms, it would seem that the ability to perceive odors from food should also be highly developed in larvae; yet we know practically nothing about the olfactory sense of larval insects.

No one, so far as the writer is aware, has ever identified organs in any larva as the ones first described in adult insects by Hicks (1857) and recently named olfactory pores by the present writer (1914a), although these pores have evidently been observed by various systematists, for Böving ('17, Pl. 118, fig. 5, *por*) has just recently seen them near the spiracles in a larva of a coccinellid beetle. Furthermore, Nagel ('94) saw two of these pores on the maxillary palpus of a larva of the stone fly, Perla bicaudata; very few on the antennæ and labrum of a lepidopterous larva of Antherea pernyi; but a few more widely distributed on the antennae, maxillae and labium of a coleopterous larva of Dytiscus marginalis. Nagel's sections through these organs must have

been poor ones, for he did not see any of the internal structures, nevertheless he called them sense organs, even though he did not observe their innervation. Since they look like pits from a superficial view, he called them "Gruben ohne Kegel" to distinguish them from similar pits bearing minute hairs. Since he failed to understand their internal structure, he did not speculate on their function.

During the past year the present writer has collected and fixed material belonging to three species of Lepidoptera. A few specimens of each one of these species have been examined, but few olfactory pores have been found. Fortunately five coleopterous larvæ belonging to the 'fig-eater', Allorhina (Cotinis) nitida L., were also collected; two of these were fixed in Carnoy's fluid and the other three were treated with caustic potash to remove all the soft tissues. Immediately after examining the integument of these larvae, it was observed that olfactory pores are quite abundant on all the appendages, and since they were easily found in sections, it was decided to use the larva of this species as a type before collecting more material in order to make a comparative study of these organs in the larvae belonging to the different orders of insects.

The larvae of Allorhina feed mostly upon the roots of grass, but sometimes they attack the roots of strawberries and other plants. The food of certain other larvae varies widely, while that of the silkworm is restricted to mulberry and osage-orange tree leaves. Since all larvae are more or less selective in regard to their food, it would seem that they have sense organs to perceive the food. The sense organs of larvae have been studied little in comparison to those of adult insects. The ocelli cannot see sufficiently well to distinguish differences between objects of the same size, because, while experimenting with the larvae of Dytiscus marginalis in water, Nagel (1894) asserts that these larvae always grabbed pieces of meat and filter paper; they immediately ate the meat but invariably released the filter paper. Nagel thought that they distinguished the meat from the paper by the sense of taste which he says is brought about by means of minute hairs (known as olfactory pegs), located at the tips of

the antennae, maxillary palpi and labial palpi. It is more reasonable to regard these hairs as tactile organs and that, in regard to aquatic larvae, food can be selected only by the insects coming into actual contact with it, because water is perhaps a poor medium for the distribution of odors. While feeding mulberry leaves to silkworms, the present writer noticed that these larvae perceived the food from a short distance and they seem to 'know' when the leaves are in their presence even though they can not see them nor touch them.

THE OLFACTORY PORES

Upon examining the integuments of the larvae of Allorhina nitida, treated with caustic potash, many minute circular light spots were seen on the head and on all of the appendages; these spots resemble hair sockets from which the hairs have been removed, but former studies dealing with similar spots on adult insects suggested that these spots might be the olfactory pores, so common to adult insects. On the last segments of the antennae, several much larger light spots were also observed; these resemble the pore-plate sense organs, common to the antennae of certain adult insects. Sections through the head and appendages show that the minute light spots are really the organs, called the olfactory pores by the writer, but instead of each large light spot being a pore-plate sense organ, it is a group of olfactory sense cells whose sense fibers pierce a thin place in the chitin, which bears all the pore apertures belonging to that group of sense cells; the thin place in the chitin may be called the plate.

In order to distinguish the olfactory pores from the second type of olfactory organ described above, the former may be called single olfactory organs and the latter compound olfactory organs.

1. Internal structure

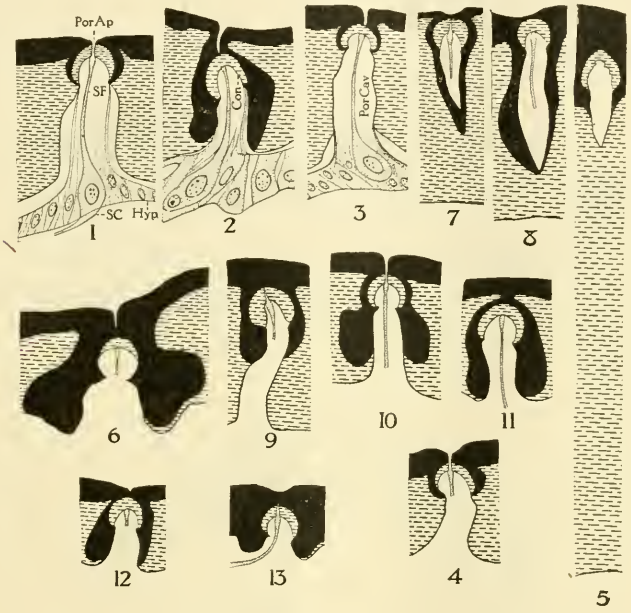
Before being able to decide definitely whether or not the minute light spots on various parts of the integument are olfactory pores or hair sockets, it was first necessary to study sections through these spots.

a. Single olfactory organs. About one half of the sections through the antennae contain no pores; in the other half of the sections it is common to see at least one pore in each section, and occasionally three pores may be observed in the same cross section. The chitin is always more or less striated and in sections stained with Ehrlich's hematoxylin and eosin, the older or outer portion of the chitin does not take the stain, but remains yellowish in color; while the newer or inner layers assume a light purple color. The thickness of the newer layers varies from two to six times that of the older ones, as may be noted in figures 1 to 15; the older chitin is represented by solid black, while the newer portion is represented by lines.

With one exception, the structure of the single olfactory organs is identical with that in adult beetles, already described by the writer ('15). This exception is in regard to the pit with which the pore aperture communicates. In adult beetles the pit is always wide and sometimes extends one-third the distance through the integument, but in the larva of Allorhina there is never more than an indication of the pit; in this respect these olfactory pores are like those in Hymenoptera, also described by the writer ('14b).

Despite the comparative thinness of sections (5 micra in thickness) this dimension is so much greater than that of the pore aperture and since the microtome knife seldom passes exactly through this opening longitudinally, it is difficult to find one of these organs which serves well as an illustration. Nevertheless, a few were observed in which the opening could be traced from the outside to where the sense fiber enters it. One good illustration (fig. 1) was found in the antenna, showing all parts of the organ, including the pore aperture (*PorAp*). Since critics have more or less doubted the existence of this opening, it was considered expedient to have a few photomicrographs made of these organs. This work was done by Mr. J. H. Paine of this Bureau. Using the ordinary photographic plates, the old chitin appears black when photographs are made, thereby concealing the pore aperture; but when a plate sensitive to yellow was used, the pore aperture was photographed quite distinctly.

In sections, more or less of the sense fiber (fig. 1, *SF*) is always present. Just before entering the pore aperture, it expands in a clublike manner; the other end of it passes through the pore cavity (fig. 3, *PorCav*) and unites with the body of the sense cell (fig. 1, *SC*), which lies in the hypodermis (*Hyp*). The hypoder-

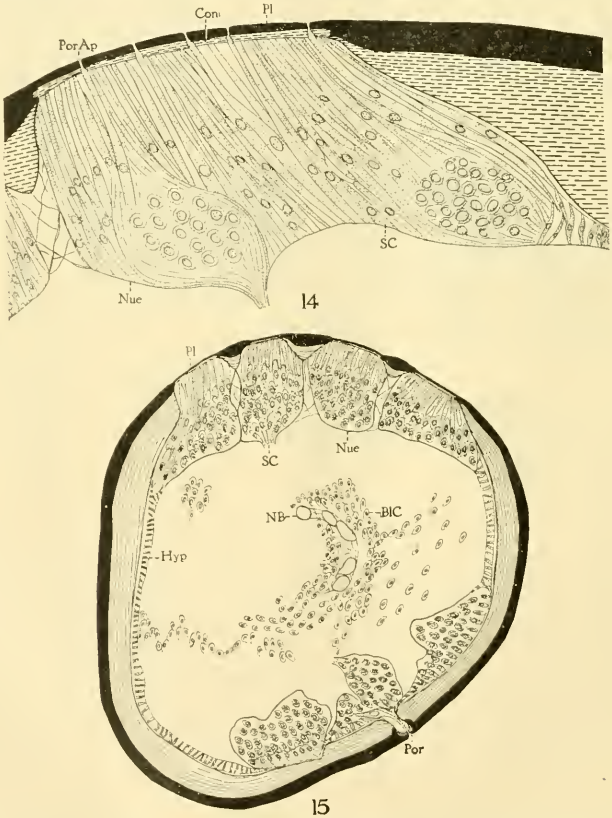


Figs. 1 to 13. Cross sections of single olfactory organs, showing internal anatomy, including darker chitin (represented by solid black), lighter chitin (represented by lines), pore aperture (*PorAp*), pore cavity (*PorCav*), chitinous cone (*Con*), sense fiber (*SB*), sense cell (*SC*), and hypodermis (*Hyp*), × 500. Fig. 1 is from antenna; fig. 2, from maxilla; fig. 3, from maxillary palpus; fig. 4, from labial palpus; fig. 5, from mandible (caustic potash preparation); fig. 6, from labrum; fig. 7, from epicranium; fig. 8, from hypopleural region; fig. 9, from coxa; fig. 10, from trochanter; fig. 11, from femur; fig. 12, from tibia; and fig. 13, from tarsus. Figs. 5, 7 and 8 were cut too obliquely to show all of pore cavities.

mis usually sends prolongations into the pore cavity and some of these unite with the bottom of the chitinous cone (fig. 2, *Con*), indicating that a hypodermal secretion passed through these prolongations to form the cone. Hypodermal prolongations have been seen in most of the olfactory pores observed in adult insects,

and it seems that the cones are a later formation than is the outer layers of the integument. In this larva the cones are usually colorless, and, when stained, take up very little of the coloring matter.

The thickness of the integument in which these pores are found varies considerably; the chitin of the mandibles (fig. 5) is 11 times as thick as that of the tarsus (fig. 13), and consequently an entire pore cavity is never seen in any one section of a man-



Figs. 14 and 15. Sections through tips of antennæ, showing internal anatomy of compound olfactory organs, including plate (*Pl*), cone (*Con*), groups of sense cells (*SC*), hypodermis (*Hyp*), neurilemma (*Neu*), nerve branches (*NB*) presumably metamorphosing cells (*BlC*), and single olfactory organ (*Por*). Fig. 14 is from a longitudinal section, × 500; and fig. 15 is from a cross section, semidiagrammatic, × 190.

dible. The chitin around the cones and pore cavities has assumed a yellowish color, causing variously shaped formations; these are represented by solid black in the drawings. The simplest formation surrounds only the cone as shown in figures 1, 3 to 5; another type surrounds all of the cone and a part of the pore cavity as shown in figures 9 and 10; and the most complex type surrounds all of the cone and practically all of the pore cavity as may be observed in figures 2, 6, 11 to 13.

b. Compound olfactory organs. In longitudinal sections through the last segment of the antenna large groups of sense cells lie in deep indentations of the chitin. At the outer ends of the sense cells the plate of chitin is extremely thin, thus allowing the light to pass through it more readily when the integument, treated with caustic potash, is examined; this explains the presence of the large light spots on the distal segments of the antennae, already mentioned. During a closer examination under the oil-immersion lens, a few minute pore apertures (fig. 14, *PorAp*) were seen passing through this thin chitin, and a thin cone (*Con*) is also invariably present. The sense cells (*SC*) are very long and slender, and are closely compact. Most of them may be cut longitudinally, but several may be cut crosswise, indicating that the latter may run to another plate. The outer ends of them are often seen running into the pore apertures, while the inner ends sometimes pass into the lumen of the antenna. The entire group of sense cells is always surrounded by a neurilemma (*Neu*).

Cross sections through the last segment of the antenna will show one or more large groups of sense cells. The cross section represented by figure 15 is typical; attention is called to the four groups of sense cells in the ventral portion (upper side of the figure) of the antenna; to the five branches of the nerve, surrounded by many small bodies, presumably metamorphosing cells, in the middle of the lumen; to the small portions of three groups of sense cells in the dorsal part (lower side of the figure) of the antenna; and to a single olfactory pore in the chitin on the dorsal side. Since thin places in the chitin are shown only at the top of this drawing, the chitinous plates (*Pl*) belonging to the sense

cells in the dorsal and ventral portions of this antenna did not lie in the same plane. It is to be noted that only about one half of each group of sense cells lies in the indentation of the chitin, and that each group is surrounded by a neurilemma (*Neu*), which, at places, unites with the hypodermis. At this place in the antenna the nerve is always divided into several branches (*NB*), and in longitudinal sections these branches may be traced to the groups of sense cells.

2. External structure

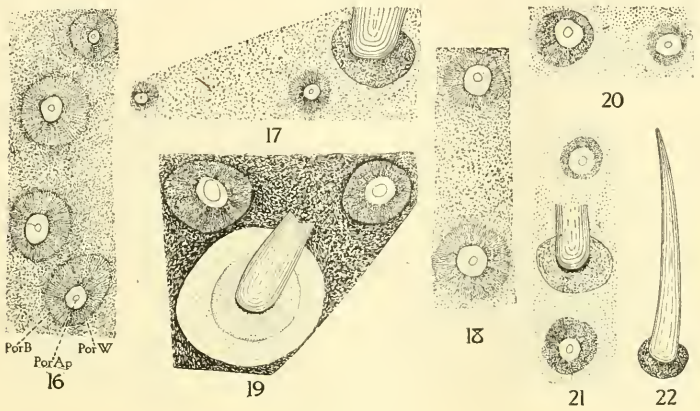
The superficial appearances of the two types of olfactory organs observed under a low-power lens have already been mentioned, and now these appearances observed under a high power lens will be described.

a. Single olfactory organs. Viewed externally under a high power lens, each olfactory pore is always surrounded by a pore border (fig. 16, *PorB*), which is usually slightly darker than the surrounding chitin, although occasionally it may be considerably darker (fig. 21). The degree of darkness of the borders seems to depend on the development of the yellowish formations around the cones and pore cavities of these organs. The hair sockets are also surrounded by borders, which are generally darker (figs. 17, 21 and 22) than the surrounding chitin, but on certain parts of the integument they may be considerably lighter in color (fig. 19). As a rule, the pore borders are striated, but the borders around the hair sockets never show striae. The striae are radially arranged, running from the pore wall to the periphery of the border (figs. 16 to 25). This is the first time that the writer has ever observed striae in the borders of olfactory pores.

The inner margin of the border is bounded by a darker line, the pore wall (fig. 16, *PorW*), which may be circular or oblong in shape. Generally speaking, the width of the pore border, the diameter of the pore aperture, and the size of the sense cell are directly proportional to the diameter of the pore wall. This means that the size of these organs, which varies considerably, is determined by the diameter of the pore wall. The chitin

inside the pore wall is much lighter in color than elsewhere, and at its center lies a minute transparent spot, the pore aperture (*PorAp*), whose diameter is diminished by focusing downward; this shows that the pore aperture is funnel-shaped.

b. Compound olfactory organs. For a general view of the plates belonging to these organs, the reader is referred to figures 23 to 25. These plates are variously shaped, but the oblong ones, as will be shown later, are the most common. Attention is called



Figs. 16 to 22. External view of single olfactory organs and hairs, × 320. Fig. 16 represents 4 organs from trochanter, showing pore border (*PorB*), pore aperture (*PorAp*), and pore wall (*PorW*); fig. 17, 2 organs and a hair from hypopleural region; fig. 18, 2 organs from maxilla; fig. 19, 2 organs and a hair from dorsal surface of labrum; fig. 20, 2 organs from labium; fig. 21, 2 organs and a hair from epicranium; and fig. 22, a hair from first antennal segment.

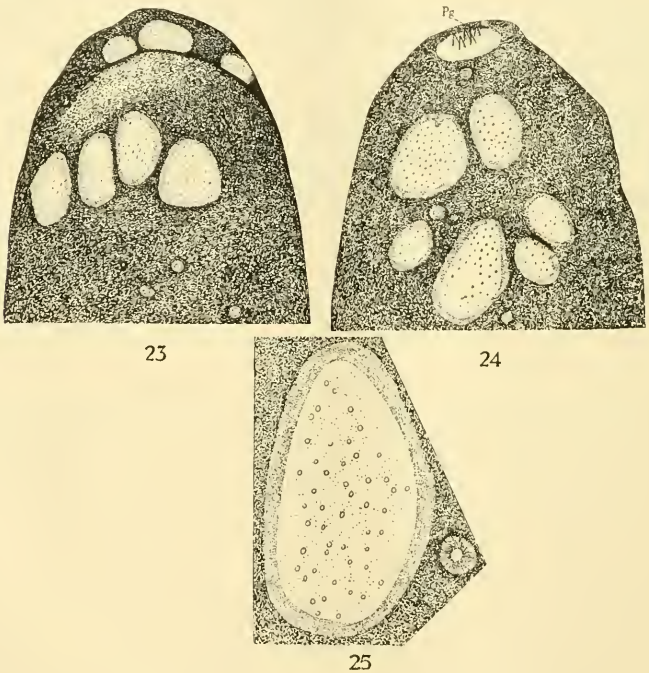
to the dark borders around the plates and to the many minute circles, the pore apertures, inside the borders.

Figures 23 and 24 are from opposite sides of the tip of the same antenna, viewed from flat surfaces, and figure 25 is the enlargement of the largest plate shown in figure 24. Attention is called to the great variation in size of these plates; on other antennae, however, the variation is even greater. It will be noted that each plate has an inside, rather than an outside border and that each one bears many pore apertures having diameters about equal to those of the single olfactory organs.

3. Disposition

In the preceding pages, the structure of the two types of olfactory organs from various parts of the integument has been described, and now the distribution and number of them may be discussed without one feeling that some of the hair sockets had been mistaken for single olfactory pores.

a. Single olfactory organs. In all of the adult insects examined by the writer, most of the olfactory pores lie in groups which are tolerably constant in number and in position, but in the larva



Figs. 23 to 25. External view of compound and single olfactory organs, and olfactory pegs on distal half of last antennal segment. Fig. 23 represents 9 compound organs and 3 single ones on ventral side of antenna, viewed from a flat surface, × 100; 2 of the compound organs at extreme tip are not drawn. Fig. 24, represents 6 compound organs, 4 single ones and 1 bunch of olfactory pegs (*Pg*) on dorsal side of antenna, viewed from a flat surface, × 100. Fig. 25 shows external structure of a compound and a single organ, × 320; the small circles represent pore apertures.

of Allorhina no groups are present, and the single olfactory organs exist only as widely separated pores; nevertheless they are fairly constant in number and in position.

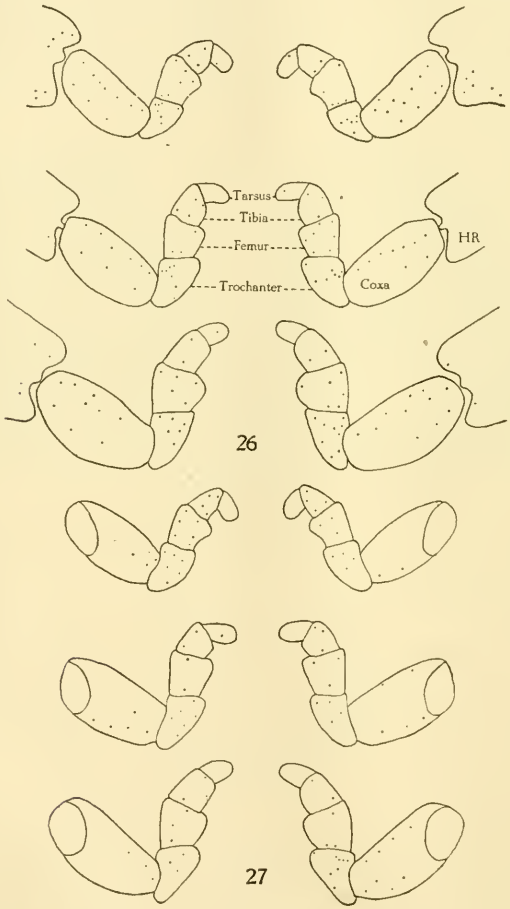
For description of the olfactory organs, the legs may be divided into inner and outer surfaces, and the other appendages into dorsal and ventral surfaces. The inner surface of the legs faces the body of the larva and the outer surface is directed from the body. Reference to figures 26 to 29 shows the disposition of the olfactory pores on one of the three specimens examined; the distribution of these organs on the other two specimens is practically the same, but the number of them varies slightly.

Each hypopleural region (fig. 26, *HR*) usually bears at least a few olfactory pores on its outer surface; these are generally near the articulation of the coxa, but sometimes one or two may be found near a spiracle which is a considerable distance from the base of the leg. Considering the three specimens examined, the pores on this part of the body vary from 0 to 12, with 6 as an average. Other parts of the body were examined, but no pores were found on them.

Each segment of the legs (figs. 26 and 27), except the tarsus, always bears at least a few olfactory pores, and the tarsus usually bears one or two. Considering the three specimens examined, the number of pores on a coxa varies from 9 to 26, with 13 as an average; on a trochanter from 7 to 14, with 11 as an average; on a femur from 5 to 11, with 8 as an average; on a tibia from 4 to 10, with 7 as an average; on a tarsus from 1 to 2, with almost 1 as an average. As an average for the three specimens examined, the outer surface of all six legs bears 132 pores and the inner surface 102 pores, making 234 pores as an average for all six legs.

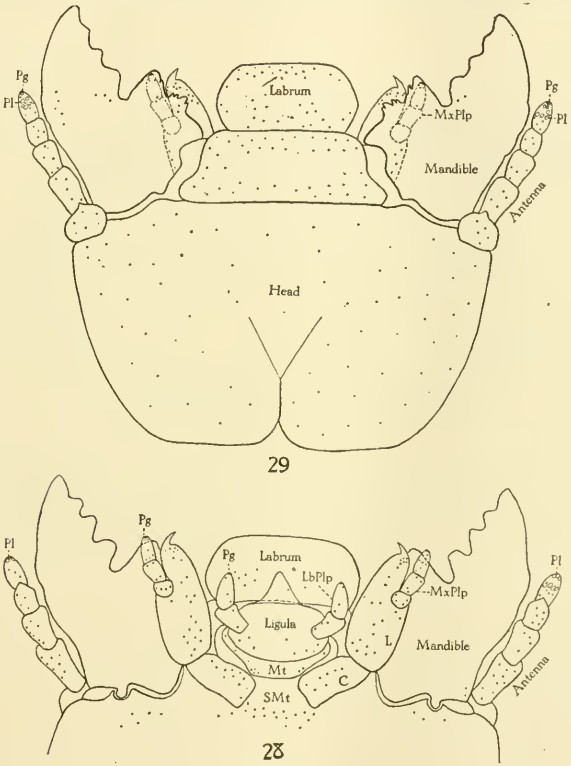
Each segment of the head appendages, and even the head proper, invariably bears at least a few olfactory pores, which are widely distributed as may be seen by referring to figures 28 and 29. Considering the three specimens examined, the number of pores on an antenna varies from 45 to 53, with 50 as an average; on a mandible from 2 to 8, with 5 as an average; on a maxillary palpus from 17 to 23, with 18 as an average; on a lacinia from 27 to 45, with 35 as an average; on a cardo from 10 to 15, with 12

as an average; on a labrum from 46 to 56, with 52 as an average; on a labial palpus from 4 to 7, with 5 as an average; on a ligula from 5 to 7, with 6 as an average; on a mentum from 8 to 12, with 10 as an average; on a submentum from 15 to 21, with 18 as an average; on the ventral side of a head from 6 to 14, with 9 as an average; and on the dorsal side of a head (chiefly epicranium)



Figs. 26 and 27. Disposition of single olfactory organs on legs and hypopleural regions (*HR*), × 9; fig. 26, outer surface and fig. 27, inner surface. The top drawings represent the front legs and the bottom ones, the hind legs. The drawings at the right in fig. 26 and those at the left in fig. 27, represent the opposite sides of the right legs, and the ones at the left in fig. 26 and those at the right in fig. 27, the opposite sides of the left legs.

from 112 to 121, with 116 as an average. All of the pores observed on the mandibles were found on the dorsal surface, with the exception of two at the base of a mandible (fig. 28) on the ventral side. Adding the average number of pores found on the three parts of the maxilla, it is thus seen that this appendage bears 65 pores, two-thirds of which lie on the ventral surface. About seven-eighths of the pores found on the labrum, all of those observed on the labium, and about one-half of those seen on the antenna lie on the ventral surfaces of these appendages. All of



Figs. 28 and 29. Disposition of the compound (*Pl*) and single olfactory organs (black dots), and olfactory pegs (*Pg*) on head and head appendages, × 9; fig. 28, ventral surface and fig. 29, dorsal surface. The opposite sides of the same appendages are shown at opposite sides in the two figures. Maxillary palpus (*MxPlp*), lacinia (*L*), cardo (*C*), mentum (*Mt*), submentum (*SMt*), and labial palpus (*LbPlp*).

the pores observed on the ventral side of the head proper lie near the bases of the antennae and mandibles (fig. 28), while on the dorsal side they are widely distributed from the labrum to the occipital foramen. For a summary of the disposition of the olfactory pores, see Table 1, page 130.

b. Compound olfactory organs. The compound olfactory organs are found only on the distal or last segments of the antennae. Generally speaking, they lie equally on the dorsal and ventral surfaces, although occasionally most of those present on an antenna lie on one or the other of these surfaces, as shown on the right antenna in figures 28 and 29. Sometimes two or three of the smallest organs lie at the extreme end of an antenna, in such a position that the plates may be seen, but the number of pores in them cannot be accurately counted, therefore they must be estimated. The plates invariably lie on the distal halves of the segments and never on the proximal halves.

Concerning the three specimens examined, the number of plates observed on an antenna varies from 9 to 16, with 12 as an average. The larger the plate, the greater the number of pores it contains (figs. 23 and 24). The number of pores in a plate varies from 6 to 68, with 26 as an average. On both antennae of specimen No. 1 (figs. 28 and 29) there are 27 plates, bearing 532 pores; of specimen No. 2, 19 plates, bearing 655 pores; and of specimen No. 3, 28 plates, bearing 687 pores. It is thus seen that on an average one of these larvae has 25 plates, bearing 625 pores. Since these pores are practically the same size as those in the single olfactory organs, their total number may be added to that of the single organs in order to compare the total number of olfactory organs in a larva with that in an adult of the same species. The writer (1915) has recorded 1,135 olfactory pores on the legs, elytra and wings of an adult Cotinis nitida, and a reference to the following table shows that the total number of olfactory pores in a larva of the same species varies from 1,254 to 1,413, with 1,359 as an average. Had the head and mouth parts of the adult beetle been examined, the total number of pores found might have equalled that found in a larva; therefore, on the basis of the total number of pores we may assume that the olfactory senses of the adult and

its larva are about equally developed, and we are safe in assuming that the adult smells well, because many species of beetles have been tested with odors (see the writer's paper on the olfactory sense of insects, 1914c).

To see how well the tip of an antenna is provided with olfactory organs, the reader is referred to figure 30, which shows in longitudinal section 5 compound organs (*Pl*) and 2 single olfactory organs (*Por*). Attention is called to the fan-shaped bunches of sense cells (*SC*) and to the manner in which the nerve sends off branches (*NB*); in cross sections these branches appear as shown in figure 15.

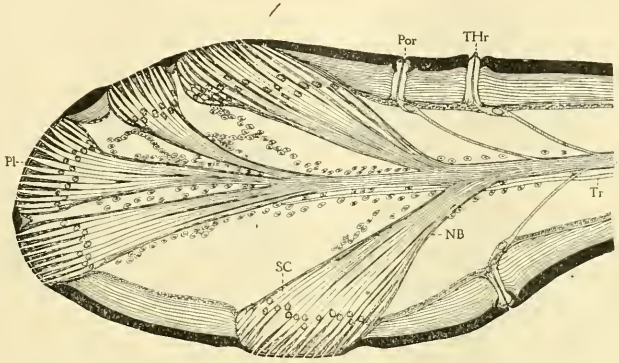


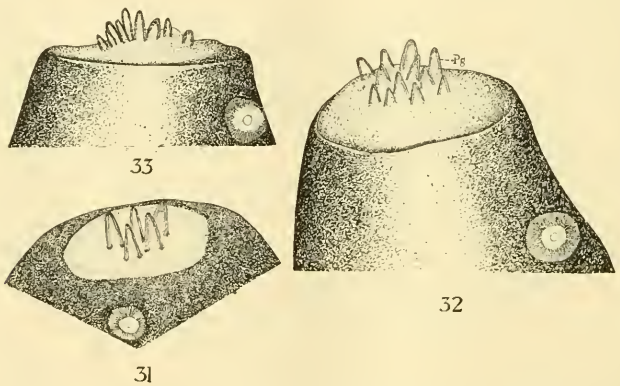
Fig. 30. Longitudinal section of tip of antenna, showing internal structure and innervation of compound (*Pl*) and single olfactory organs (*Por*), and tactile hair (*THr*); two-thirds diagrammatic, × 100. At this magnification the pore apertures are never discernible. Sense cell group (*SC*), nerve branch (*NB*), and trachea (*Tr*).

THE OLFACTORY PEGS

Short, stubby hairs, known as olfactory pegs, are common on the antennae and mouth parts of most adult insects. They may or may not arise from pits, but are always innervated. Since their tips are covered with hard chitin, they certainly can not serve as olfactory organs, although they are well adapted for tactile purposes.

While hairs are common to all the antennal segments of adult insects, they were formed on only the first and last antennal segments of the larva under discussion. The only hairs present

on the first segments are long and rather stout (fig. 22); they are not innervated. On the last segment two types of pegs were found; one type arises from pits and the other type from thin chinitous plates. Of the first type only one hair was observed (fig. 30, *THr*), but a bunch of hairs belonging to the second type was found at the extreme tip of each antenna, maxillary palpus and labial palpus (figs. 28 and 29, *Pg*). Each bunch of hairs arises from the center of a thin, transparent plate. On the antenna the bunch consists of about 5 hairs (figs. 24 and 31, *Pg*); on the maxillary palpus of about 12 hairs (fig. 32); and on the labial palpus of about 9 hairs (fig. 33). The walls of these hairs are



Figs. 31 to 33. Disposition of olfactory pegs (*Pg*) and single olfactory organ at tips of three head appendages, × 320; fig. 31, dorsal surface of antenna; figs. 32, dorsal surface of maxillary palpus; and fig. 33, ventral surface of labial palpus.

comparatively thick, and for this reason such organs are not well adapted to receive chemical stimuli, nevertheless Nagel (94) called them gustatory organs in the larvae of Dytiscus marginalis, for he asserts that these larvae are able to distinguish meat from filter paper only after having brought these hairs into actual contact with the objects dropped into the water. Being ignorant of the location of the olfactory pores in these larvae, Nagel naturally assumed that these hairs are organs of taste; nevertheless, it seems more reasonable that aquatic larvae can distinguish food only by actually coming into contact with it, for water is perhaps a poor medium for the distribution of odors.

The writer did not spend any time studying the innervation of the hairs under discussion, for vom Rath ('88) has already determined that each bunch of hairs is provided with a sense cell group, from which runs a sense fiber to the base of each hair.

SUMMARY

So far as known to the writer, the two types of olfactory organs herein discussed are reported for the first time in a larva. The single olfactory organs have been seen by various observers, but have never been identified as the organs, called the olfactory pores by the writer. The compound olfactory organs have certainly been observed, but have probably been regarded as the pore-plate sense organs, so common to the antennae of adult insects.

In regard to structure, the olfactory pores are like those in adult beetles, with the exceptions that their pore apertures do not communicate externally with pits and that the borders surrounding the pore walls are striated. The compound organs are patterned after the single organs, but since the available space inside the tip of an antenna is limited and as the integument must not be materially weakened, the following two modifications are present; 1) the sense cells lie in closely compact groups, thereby causing a long and very slender form, whereas those belonging to the single olfactory organs are shorter and much larger in diameter; and 2) many pore apertures lie in the same plate, more closely together than would be possible for the pore apertures belonging to single organs. By this means the least possible amount of the integument is devoted to pore cavities, thereby weakening the integument as little as possible, and at the same time rendering a larger number of pore apertures possible.

The single organs are more widely distributed in the larvae than in the adult beetles; in the former they are found on the head, on all of the head appendages, legs and hypopleural regions; and in the latter on the elytra, wings and legs, and probably elsewhere, although they have never been looked for on other parts of the integument, but it is unlikely that they will ever be found on the antennae and thorax.

The compound organs are found only on the distal halves of the last antennal segments.

Since the pore apertures in the compound and single organs are practically the same in size, it may be assumed that a sense cell belonging to a compound organ functions equally as well as one belonging to a single organ; and since an adult beetle and its larva have practically the same number of pore apertures, it may further be assumed that the larva smells equally as well as the adult form, and we know that adult beetles smell well.

TABLE 1

Disposition of olfactory pores observed on three larvæ of Allorhina (Cotinis) nitida

NUMBER OF SPECIMEN	COMPOUND ORGANS			SINGLE ORGANS									TOTAL NUMBER OF PORES
	Antennae			Mouth parts						Head	Thorax		
	Plates of compound organs		Single organs	Mandibles	Maxillae	Labrum	Labium	Mentum	Submentum	Epicranium	Hypopleural regions	Legs	
	Number of plates	Number of pores in plates	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	Number of single pores	
1	27	532	95	10	139	46	18	10	15	121	25	243	1,254
2	19	655	106	9	113	56	15	8	21	129	51	250	1,413
3	28	687	102	10	141	54	15	12	18	122	37	210	1,409
Average number of pores	25	625	101	10	131	52	16	10	18	124	38	234	1,359

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STUDIES ON CELL DIVISION IN THE ALBINO RAT
(Mus norwegicus albinus)

III. SPERMATOGENESIS: THE ORIGIN OF THE FIRST SPERMATOCYTES AND THE ORGANIZATION OF THE CHROMOSOMES, INCLUDING THE ACCESSORY

EZRA ALLEN

Department of Zoology of the University of Pennsylvania and the Laboratory of the Wistar Institute

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1. INTRODUCTION

Purpose and scope of the study. On account of difficulties experienced in fixing mammalian material, great disagreement exists in the literature on mammalian spermatogenesis, not only concerning the number of chromosomes, but also concerning such important points as the presence and nature of an accessory, and the forms, constitution, and history of the individual chromosomes. My study on the germ cells of the white rat was begun with the hope of solving some of these problems for one mammal. The results recorded in this paper set forth the basal number of chromosomes, the presence of one accessory, and the shape, organization, and behavior of most of the chromosomes throughout their history in spermatogenesis; the development of the first spermatocyte cells from spermatogonia; also a statement of the problems with respect to the origin of spermatogonia and Sertoli cells, and the cytoplasmic bodies known as the idiosome and the chromatoid body. Spermiogenesis is not discussed.

2. MATERIAL AND TECHNIQUE

Material. The rats came from the colony of The Wistar Institute of Anatomy and Biology. Only the standard strain of Albino was employed. Over one hundred individuals contributed to the study, aged from birth to full maturity. Most of them were used in obtaining a satisfactory method of fixation and preparation.

The traditional fixatives and technique had proved inadequate in the hands of previous workers and were equally so in mine. After experimenting with practically every well-known fixative

and various modifications of them, the following fluid gave the most satisfactory results:

Picric acid, sat. aq. sol.	75 cc.
Formol (c. p.)	25 cc.
Glacial acetic acid	5 cc.

To this mixture (Bouin's fluid), freshly made and raised to a temperature of 38°C., are added and thoroughly dissolved the following, in the order stated: 1.5 grams chromic acid crystals, and then 2 grams of urea crystals. Each of these latter should be dissolved as added, or a precipitate will be formed when the urea is put in. Even then, unless the formol is of a high degree of purity, this white precipitate is likely to occur. In small quantity it does no harm. In passing, it is well to note that all the chemicals should be the very best obtainable.

Technique. From an animal that has been killed either by chloroform or by decapitation, the testis is removed as quickly as possible and snipped into small pieces, which are dropped into the fixative as they are cut. The fixative is at the temperature of 38° or 40°C. and is held at that temperature during the first hour of fixation. Fixation is complete in from one to two hours, depending upon the age of the animal. Longer fixation, up to forty-eight hours, although accompanied by more general shrinkage, does not seem detrimental to the chromosomes.

The fixative is replaced by the drop method with 70 per cent alcohol, the picric acid washed out by the addition to the alcohol of a saturated solution of lithium carbonate added a few drops at a time; the alcohol is replaced by anilin oil (freshly distilled), this by synthetic oil of wintergreen, and this by paraffin of 52° melting point. The oils are changed by the drop method. The paraffin is added very slowly until the tissue is in a bath of high paraffin concentration. It is then passed through several changes of paraffin to remove all of the oil, a process which takes from one to three hours, depending upon the size of the pieces of tissue. All details of the method, which is very simple, are recorded in my paper upon the study of the effect of fixatives (Allen, '15).

Sections were cut at thicknesses of from 2 to 10 micra. Those found most valuable for study were from 5 to 7 micra. The very

thin sections did not add materially to the ease of studying the individual chromosomes, but were found useful in making some of the photographs, as noted under explanation of figures. As nuclear stains, iron-alum-haematoxylin and safranin were employed; the counterstains were orange G., methyl green, and acid fuchsin. Flemming's triple stain does not yield very satisfactory results after any picro-formol-acetic fixation unless the slides are soaked in 50 per cent hydrogen peroxide from five to ten hours; but even with this treatment I did not find the stain very satisfactory upon the rat testis. The most satisfactory stain for chromosomes, whether used after Flemming's fluid or the picro-formol-acetic-chromic-urea fluid (described in my paper to which reference has been made as B-15), was iron-alum-haematoxylin without counterstain.

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3. OBSERVATIONS

The Structure of the Rat Testis

The testis of the rat is typical of the mammals. In the adult, each tubule is constantly active in the production of spermatozoa up to old age. This production of the mature spermatozoa is a wave-like activity passing along the tubule, so that in one part of the wave dividing spermatogonia and nearly mature spermatozoa appear, while in another part early stages of first spermatocytes and spermatids occur. The latter condition is shown in figure 1. In other parts other combinations of the various stages are to be seen. Von Ebner ('88) worked out this cycle in the rat, finding it to occupy 32 mm. in length. That is, in a given tubule, dividing spermatogonia are found in localities separated by 32 mm., of the tubule length. Similarly, first spermatocyte metaphases, or other definite stages, repeat themselves along the tubule at this distance, and in each case the other cells show their corresponding stages. Two portions of the wave are shown in figures 1 and 46.

The Spermatogonial chromosomes

Figure 16 is drawn from a cell showing 37 chromosomes in metaphase. They are all rod-shaped, slightly curved, and of about equal diameters. I have not been able to differentiate an accessory in the spermatogonial complexes, although many cells have been examined with this in mind. The cell figured is taken to be typical with respect to number. Additional evidence for this number consists of another cell divided between two sections which shows clearly 31 chromosomes in one section and 8 in the next; and of a few more cells which show in each case clearly 30 plus some more which are too intermingled for accurate counting; also of a cell in the cerebellum of a male rat which shows clearly 37 chromosomes. In no case has a count yielded less than 30 plus some indistinct ones, neither has there been positive evidence to indicate a greater number than 37. The haploid number strengthens the acceptance of 37 as the basal number, as there is abundant evidence for fixing this at 19 including the single accessory. The small size and the strong tendency of the chromosomes to lie in more than one plane closely overlapping each other are two conditions which have proved serious obstacles to accurate enumeration.

As seen in the figure, the chromosomes differ widely in lengths —14 long, 13 medium, and 10 short. The dividing line between the second and third groups is less distinct than between the second and first. The chromosomes marked *A* are the longest pair and can be followed through other stages. The difference in size between the second and third largest pairs is not so great, and consequently they are difficult to determine accurately. All are more or less curved. In the young testis they are longer, less dense, and more scattered than in the adult. Many adult cells were studied, but not one was found which was satisfactory for an accurate count. I have not attempted counts in the late prophase stages because the chromosomes are seldom in such position as not to obscure each other in some degree, although it may be possible to use this stage for number determination.

Figure 17 shows that division is normal, i.e., longitudinal. The fiber attachment is terminal.

The history of the chromosomes during the first spermatocyte changes

This portion of the paper will be divided into two parts: 1) The early period of development, up to the stage when the chromosomes are ready to assume the characteristic tetrad forms, or the diplotene stage, and, 2) the later period, covering the changes occurring from the beginning of diakinesis through the stages commonly known as prophases, and up to their metaphase and first maturation division.

The rats which furnished the material for the early period were aged, respectively, 7, 10, 11, 12, 13, 14, 15, 16, 20, 24, 29, and 35 days after birth, each from a different litter. This series enabled me to identify positively each stage in the early development of the first spermatocyte chromosomes. The results are recorded in figures 2 to 15.

For the study of the later stages, testes from two rats aged 37 and 50 days after birth, respectively, were the most serviceable. Material of this age seems to fix better than that which is much older, although in one case exceptionally good conditions were found in a rat aged 180 days. Ripe spermatozoa are to be found in the lumina of the tubules of 37-day rats, so that spermatogenesis is complete at that age. Other testes from older rats contributed also, but to much less extent than the two first mentioned.

1. The early period. These stages are illustrated in the series of figures in plate 2. The cross-sections of the tubules show the changes which take place in the development of the spermatogenic layers. Since these are all photographed at the same magnification, they show the diameter changes incident to growth as well as the arrangement of the cells. The more enlarged figures show the cells in greater detail in their progressive development.

Preleptotene stages. The cells in figure 2, from a 7-day testis, are apparently undifferentiated. The nuclei lie in a syncytium which fills the tubule. For convenience I call these cells Type A. Two of them are seen dividing (*D.C.*, the left-hand one out of focus). The larger, round cells in which the nuclear plasm is less dense are preparing for division. These cells of type A appear again clearly in the other figures.

Figure 3, from 11-day-old material, shows that two distinct changes have occurred, in addition to a slight increase in the diameter of the tubule. These are 1) a marked increase in the number of the cells and, 2) the presence of a new type of cell, which I call Type B, seen more enlarged in figure 9. It is characterized by its spherical form and the densely staining, sharply-defined masses of chromatin. Its origin is shown in figure 5. The cells marked *D.C.* are cells of type A in division. Two characteristic daughter cells appear at *B*. They are plainly type B, though small. They grow rapidly in size, and since many are produced simultaneously they may form practically all of the outer cell layer, as in figure 6. The orientation of the division plane may be as shown in figure 6 or as in figure 5—either longitudinal or radial. In either case the cells soon migrate inward, as seen in the upper part of figure 7. The lower part of that figure and figures 5, 6, and 8 show that the subsequent growth changes occur at deeper levels, or nearer the lumen.

Cells of type B transform to cells of type C (figs. 4 and 10) by the chromatin masses becoming more diffuse, which thus lose their sharply defined edges, as seen in section, and assume a woolly appearance.

The next stage of development is seen in figure 11. Here the spireme has evidently begun its differentiation, as seen by the presence of a thread made of small, woolly, lightly-staining chromatin masses. These by approximating toward each other, and condensing, pass to the leptotene stage through the condition shown in figure 12.

Leptotene stage (fig. 13). The lower part of figure 7 shows this condition of the tubule when the tissue is not sufficiently destained, as the nuclei reveal no structural details unless destained to a point at which the cells of type A have lost all color. The spireme in this, as well as in the stage shown in figures 11 and 12, is apparently of the segmented type, though perhaps determination of this point is questionable owing to the small size of the cells and the large quantity of chromatin present. The leptotene stage is characterized by the extreme fineness of the thread, which now appears narrowest in its history, yet stains deeply.

Zygotene stage. Following these stages just described, synapsis occurs. Again the densely packed spireme makes observation difficult. With care one finds the condition shown in figure 15, indicating para-synapsis, union occurring at one end first. A very slight polar aggregation may occur during this period, but no synizesis has been observed with any method of fixation.

Pachytene stage. The completed process of synapsis gives us the pachytene condition, (figs. 5, 8, 14, and fig. 1, P.c.). This stage is recognized by the greater spaces between the spireme threads and, in favorable places, by the double nature of the threads. The union is extremely intimate, so that this double nature is seldom visible except in the early stages. It is in this stage that the cell first shows complete differentiation of the cytosome. In adult material the syncytium has ceased to exist and the spermatogonia are consequently individualized. In the young material this differentiation of the cell is always preceded or accompanied by the appearance of the lumen in the tubule, as shown in figures 5, 6, 7, and 8. The lumen in the series studied appeared first in the testis of the 14-day rat, not at all in the younger testes.

Conclusions. It appears that the first spermatocyte cells begin their differentiation with the organization of the nuclei in the daughter cells of the last division of an undifferentiated type of cell (Type A), which is the only type then present in the tubule. This process begins between the age of 7 and 10 days after birth. The pachytene stage is reached by the age of 14 days. For further considerations see the discussion, p. 160.

2. The later period. As stated on page 138, the material for this part of the study is mostly from rats aged 37 and 50 days. Older material was compared with it and found essentially similar though not always so workable. The material younger than 37 days helped in understanding the first changes which occur in early diakinesis.

The changes from the pachytene condition to the formation of tetrads and the organization of the different forms of spermatocyte chromosomes are difficult to follow for each chromosome. However, the various stages in their development are readily com-

parable with animals in which the chromosomes have the same final shapes, the histories of which have been worked out (e.g., the Orthoptera; McClung, '14, and Wenrich, '16), so that one may say that the history of the rat chromosomes during this period corresponds with that of the similarly shaped and constituted chromosomes of those forms which are so much more favorable for study. This topic is further treated under the discussion, page 159.

Diakinesis. The beginning of differentiation into the shapes assumed by the chromosomes is seen after the diplotene stage has passed and diakinesis is occurring. Threads begin to separate longitudinally, when either the ends remain attached (fig. 34) or spread apart (figs. 30 and 31). In the latter case adherence is maintained near the ends. Single, double, and even more complex rings (figs. 27, 30, and 34) are thus formed. The threads are delicate in structure, with the chromomeres quite well separated, as shown in the photographs, figures 47 and 48. At the places where the threads in the double rings apparently cross, their duplex constitution may sometimes be made out, as illustrated in figure 34. The tetrad condition of the chromosome is thus evident in the early prophase. Later stages confirm this evidence. The exact time when the longitudinal splitting of the homologous chromosomes takes place has not been determined. It seems to be accomplished by the time that the shapes are established.

Further treatment of the changes occurring in the later period follows under the topics: 'The shapes of the first spermatocyte chromosomes,' 'The composition of the first spermatocyte euchromosomes,' 'The number and size of the first spermatocyte chromosomes,' and 'The accessory.'

The shapes of the first spermatocyte chromosomes. The only shapes assumed are the rod, the cross, and the various types of the ring—single, double, and triple. These are shown in figures 18, 19, 27, 28, 29, 30, 31, 50 to 52, 54 to 57. Some interchange seems to occur in the number of rings and crosses, indicating that the individual chromosomes do not always assume the same final shapes. Thus in some cells in prophase two crosses of nearly

the same size are clearly present. In others, no large cross is decipherable, but a small one appears. In two cells I saw one large and one small cross. The metaphase complex in lateral view shows a cross sometimes (fig. 57) and again none, but at this stage the cross is very difficult to differentiate unless viewed *en face*. Two are present in the cell shown in figure 27, a large and a small one. This cell has sixteen rings (one not drawn), two crosses, and one rod, the accessory. The curved rod is the constant shape of the accessory after differentiation. (Figs. 27, 18, 19, 20, 21, 22, 54, 55, 56, and 58).

The largest chromosome, *A*, shown in figures 27, 30–33, seems variable between the double and triple form of ring. Its triple form is often seen in the various prophase stages, and again, as in figure 27, it is found in the double ring form, with a delicate bridge of chromatin connecting the sides of the larger loop and a more delicate one between the sides of the smaller loop (the upper one in figure 27). Chromosome *A* in its triple form has been noted four times in the same cell with chromosome *B* in double ring form. The small double ring shown in figures 27 and 50 (from different rats) is very common.

The sketch of the chromosomes appearing in figure 27 is, as stated above, made from one cell. One chromosome, completing the full number 19, is not shown because its form is not positively determinable. It appears like a double ring seen on end, rather than a cross, and since no other shape but rings and crosses (aside from the accessory) has been found in all the rats studied, it is fair to assume that it is a ring lying in such a position as to render its exact outline doubtful. This assumption is strengthened by the fact that in a near-by cell *three* double rings and chromosome *A* in its triple form are present. In another, a small double ring with widespread lugs is to be seen, which would correspond in size with the one in question. It is drawn in figure 28. It is not likely that this doubtful chromosome is *A*, because it is too small, and, in addition, it has stained much more deeply than *A* usually does at this period. Furthermore, the chromosome marked *A* in figure 27 is less dense than the others, indicating in that respect its similarity to the normal condition

of *A*, which is usually retarded in its differentiation. It is possible that the obscure chromosome is *A* in an abnormal condition, but this hypothesis is only suggested.

No other cell found showed its chromosomes clearly enough for an accurate identification of more than thirteen out of the full number of nineteen present.

The high percentage of rings is always manifest. The intermediate-sized rings are so nearly of the same size that their individual measurement is very questionable. It would probably be possible to differentiate them were they all to lie perfectly flat, a position which, unfortunately for this purpose, they do not assume. The exact size relation is not shown in figure 27, since some are inclined or curved to the plane of drawing. *B* is actually larger than it appears in the figure. *A* is always considerably larger than *B* (perhaps one should say longer), and probably for that reason lags behind the others in its condensation, a circumstance which tends to over-emphasize its actual size when measured in quantity of chromatin.

The chromosomes marked *A*, *B*, *C*, and *D* in the various figures are recognizable in different stages. *B* and *C* resemble each other after early prophase. Chromosome *C* may be early identified by its strongly elliptical outline and open ends, as in figure 27. Whether it originates as a double ring like *B* is still a question, as I have not seen two large double rings in any cell in which *A* is present in its triple-ring form. Chromosome *D* is early identified by its widespread lugs at one side and closed-ring formation at the other, as shown in figure 27. This chromosome is troublesome in polar views of metaphase plates, as may be judged from its form and from its appearance in figures 18 and 19. Chromosome *C* is marked doubtful in figure 19. It is either a cross or a ring with widespread lugs, the parts in such position as to obscure the opening. If a ring, it is identifiable as chromosome *C* by its size.

The identity in late anaphase of chromosomes *A* and *B* is of course doubtful, as they are both large. Figures 39 to 42 would do as well for *A* as for *B*. The size is the only distinguishing character in these stages. Likewise *C* and *D* in similar stages

would be indistinguishable, as their size is so nearly equal. Chromosome *E* has been seen in many cells. Whether its form is constant, or whether it may assume the form shown in figure 28, is a matter that has not yet been determined.

The other groups of rings of medium and of small size are not to be followed individually for similar reasons. The two large crosses are in the same category. The accessory, however, always stands out distinctly. Its history is treated separately on p. 151.

The constancy of form of all, or of the principal chromosomes, is too large a topic for complete discussion in this paper. Aside from the evidence cited for *A* and *B*, my observations lead me to conclude that such constancy is doubtful. Numerical data of large numbers are necessary to establish this point. Whatever such study may show, my observations upon hundreds of cells leads to the conclusion that the shapes assumed are limited to the various types of ring (as seen in figures 27, 28 and 31), the cross, and, in the case of the accessory only, the curved rod.

The composition of the first spermatocyte euchromosomes. This topic is closely related to the former in that it treats of the organization of the shapes just described. Briefly stated, this organization is typically tetrad, with the sole exception of the accessory, which is a diad, and is treated elsewhere as a heterochromosome (p. 151). By 'typically tetrad' is meant that there are four elements in each chromosome, each of which is the product of the longitudinal splitting of the respective paternal and maternal chromosomes which paired in synapsis. McClung's name for these elements, 'chromatid,' will be used in this paper. His interpretation of a chromosome as the unit mass of chromatin which divides in cell division is also retained in the discussion of the spermatocyte euchromosomes, although some writers have preferred to think of these chromosomes as pairs of chromosomes and so to denominate them.

The tetrad composition is clearly seen in the large and medium-sized chromosomes. In the case of the simple rings it appears clearest in the midprophase conditions (fig. 27), but it is to be seen in the large double ring in diakinesis (fig. 34). These larger

members of the complex show their composition also in the metaphase, after the chromatin has become homogeneous or nearly so. The crosses differentiate their four chromatids in prophase (fig. 29) and in metaphase (fig. 57). The small rings of the complex are not large enough to decipher minutely, but judging from their appearance as outlined in figure 27, we may conclude that they are not exceptional in organization.

Further proof of the tetrad composition is found in the anaphase forms. These are simple V's. They are shown in figures 22, 41, and 42. The large ones are readily seen. The smallest are obscure, but their V outline is often discernible, as shown in figure 22.

The history of chromosome *B* is illustrated in figures 34 to 42. The account must begin with diakinesis, as so far I have been unable to trace any pair of chromosomes through the synaptic period. The critical point in diakinesis is of course the observation of the longitudinal split in the original pair of chromosomes. That this split has occurred is shown in figure 34. The same figure shows also the arrangement of the chromatids in the tetrad, or the architecture of the tetrad. It is evident that there is no crossing of the chromatids. While this tetrad does not show throughout its extent this longitudinal split, the arrangement of the chromatids is sufficiently seen to demonstrate, from what we know of this same form of chromosome in forms more favorable for study (the Orthoptera, for example), that the tetrad formation has been accomplished. The two portions of the double ring lie at right angles to each other. The side of the larger is divided and each member of this division passes to opposite sides of the smaller ring. This constitution is maintained throughout the first spermatocyte phase, as seen in figures 35, 36, 37, 18(A), 40, and 51. The architecture is shown diagrammatically in figures 43 to 45, taken from McClung ('14). The knobs in figure 35 represent the fused ends of the chromatids. The elliptical ring appearing in this figure is formed of two chromatids with their ends thus fused. The mirror image of this ring lies behind, one end seen at the right. A side view is given in figure 36, which shows that the fused ends have diverged in pairs,

the right-hand pair more than the left, but that union is still preserved along a large portion of the structure. This arrangement of parts persists while the chromatin becomes steadily more condensed and the chromosomes assemble to form the metaphase plate. The appearance of chromosome *B* is shown in figure 37 in partial side view, and in figure 18 viewed from above.

The history ends with division, by which this tetrad is separated into two simple V's on the spindle, as shown in figure 42. Between this stage and that shown in figure 35, the movements of the chromatids have been as follows: the sides of the ellipse have approximated along their whole length, the spindle fibers have been attached at the ends (on the right in the figures), and the tetrad has stretched out gradually along the spindle, assuming progressively the forms shown in figures 37 to 40. Figure 40 shows almost the last stage previous to division. Just following this condition the hump disappears completely and the chromosome seems to be a single rod. Its composition cannot be discerned, but just as soon as the central ends separate, the V's appear. At first their sides are very closely approximated, as shown in figure 41, but they quickly spread apart and appear as in figures 42 and 22.

The history of this chromosome may be regarded as typical of all the ring-shaped forms. The more complex ring and the simple rings vary in slight details only. The chromatid arrangement in the triple ring has not been seen in diakinesis, but as indicated by figures 18, 19, 32, 33, and by figures 40 to 42, (the last three serve as well for this chromosome as for *B*), there is no reason why it should be interpreted differently. The simple ring is illustrated in figures 18 (*D*), and 27 (*D* and several small ones).

Figures 27 (C), 35, 37, 39 and 51 are typical of the large ring in which the pairs of chromatids separate at both ends. It would seem that in some of the medium-sized and smaller rings this double separation does not occur, as the lugs are seen at only one end. (Figs. 18 and 27).

In the case of the cross, the V which results in anaphase is formed by the separation of two pairs of arms, shown in figure 29

and figure 57. This arrangement on the spindle may apparently be either as shown in figure 57 or in the position of having rotated 90 degrees, so that a lateral view of the spindle reveals this chromosome as a ring still having its knob, or the appearance given in figure 40. This position is found in oblique views of the spindles so cut as to have removed some of the chomosomes. When all are present, these refined observations cannot be made since the various members of the complex lie so closely together. The polar views of the cross in metaphase are even more troublesome. (See notes on figure 18 under description of figures.)

I have had no opportunity to trace the early history of the formation of the cross. Until such observation is made I am inclined to accept its formation as that shown by McClung ('14) and Wenrich ('16) for the cross in Orthopteran complexes.

That the small rings are also tetrad in formation must be accepted on the evidence shown in figure 27. Mention may also be made of the fact that none but small rod-like forms, with or without humps, appear in lateral views of the metaphase complexes. In polar views these small chromosomes appear round or oval, as in figures 18 to 20. The triangular-shaped masses in anaphase, although too minute to show the arms of the V, furnish additional evidence that they are of similar organization with the larger rings.

The large chromosomes are always the last to divide. They are to be seen in late anaphase stretching along the spindle between the chromosomes that have divided and are assembling at the pole. Sometimes three or even four are thus found. The last two to divide are *B* and *A*. The appearance of one of these is shown characteristically in figure 22. Others present in this particular cell were not drawn.

Two points of interest remain to be noted. First, the terminal attachment of the spindle fiber is common to all of the chromosomes, as it was in the spermatogonia. This fact accounts for the simple V's in anaphase rather than the double ones seen in such forms as the Stenobothrus type described by McClung ('14). The second point is that no V's have been seen in polar views at metaphase. The V-formation occurs at this stage in the

Hippiscus type (McClung '14), and is explained as failure of the rings to form completely, or to persist as such. Such failure apparently does not occur in the white rat. The polar views shown in figures 18 to 20 are to be regarded as typical in this respect.

After division takes place the movement in anaphase is rapid, the small and medium-sized chromosomes aggregate close together quickly, but do not lose their identity until after the larger ones have begun to approximate the pole. Such a well-separated complex at this stage as that shown in figure 22 is rather rare. On account of this rapid movement, as well as the strongly convex telophase plate, study of the fate of the V's is very difficult. They do not reappear as V's in interkinesis nor in the second spermatocyte complexes. We may only assume that their duplex constitution is retained throughout interkinesis.

Conclusions as to the constitution of the first spermatocyte euchromosomes:

1. Whatever the shapes assumed, they are all tetrads formed of four chromatids.

2. Their organization is such that each chromatid is so related to the others that it may maintain its individuality throughout the various movements which occur from the beginning of diakinesis until the separation in pairs is accomplished at anaphase.

3. The fiber attachment is terminal, so that only single V's are formed in anaphase.

4. Ring formation is always complete, since no V-shaped tetrads have been observed in metaphase conditions.

3. The number of the first spermatocyte chromosomes. Determination of the number has been difficult for several reasons. The chromosomes do not form flat plates, they divide non-synchronously, the largest one (*A*) is frequently late in taking its place on the spindle, as shown in figures 46 and 54. These facts render polar views difficult to interpret. Certain false interpretations are likely to arise by counting lugs separately, as in four of the largest chromosomes they spread very widely. Another polar-view difficulty arises from the presence of crosses. It is possible so to section these that the middle parts as they lie on the spindle may appear as two small chromosomes lying in the same plane.

In lateral views of the spindle the chromosomes cannot be enumerated if the sections are cut thick enough to include them all, since they lie close together, and some are within the outer layer, as is seen in figures 18 to 20. Furthermore, many are about equal in size and shape, so that there is no means of identifying particular ones except in a few cases, as already indicated. Still another difficulty is presented by the nucleolus. The fate of this body is doubtful. The evidence in the rat so far gathered points to a gradual absorption, as discussed under the topic 'The nucleolus,' p. 151. The faintly staining body in figure 18 may, with heavy staining, look exactly like a chromosome.

The only place where one may be perfectly confident of counts is in late prophase, just before the chromosomes assemble on the spindle. They are then scattered and distinct. It is upon counts made at this stage that I rely chiefly for my decision that 19 is the haploid number of the chromosomes. Thirty-one entire nuclei in which the chromosomes could be enumerated gave the following results:

24 cells, unquestionably 19 chromosomes and a nucleolus
5 cells, unquestionably 19 chromosomes, no nucleolus
1 cell, unquestionably 20 chromosomes and a nucleolus
1 cell, unquestionably 18 chromosomes, no nucleolus

It is to be seen that these thirty-one cells give an average of 19 chromosomes. Added to this evidence are five equatorial plate counts, one of which is from a smear preparation, each giving 19 chromosomes. Three of these are shown in figures 18 to 20, all from different animals. As still further evidence, one cell in anaphase showed 19 chromosomes at one, and 18 at the other pole.

Under the topic dealing with the shapes of the chromosomes it was noted that 16 rings had been found in one cell, all but one being shown in figure 27. Thirteen of these are to be seen in metaphase in figure 18, with a possible 14th—*C*,—if that be a ring. Eleven rings are clear in the cell from which figure 19 was made. The metaphase plates have not revealed more than 14 rings. It would therefore appear that we may assume 16 rings as probably the highest number of rings usually occurring,

the other two tetrads being crosses, especially in view of the fact that not more than two crosses have been seen in any one cell. However, this statement is not to be interpreted as excluding the possibility of the occurrence of more rings and fewer crosses, or vice versa, in any one cell.

4. The sizes of the first spermatocyte chromosomes. When classed according to size, these chromosomes form three groups, more or less well differentiated: 5 small, 7 intermediate, and 7 large. These may be noted in the polar views, figures 18, 19, and 20; also in figure 27. The seven large ones appear readily distinguishable in figures 19 and 20. In figure 18 the lines between the three groups are very difficult to draw. In figure 27 the seven large ones may be distinguished. But the line between the intermediate and small is always difficult to establish.

While the accessory would appear in the equatorial plate views to belong to the large group, one needs to remember its less dense condition and its curved-rod shape, the curve of which is not as strong as that of the rings. Moreover, the ring chromosomes are extended in two directions by the lugs, the lengths of which do not directly appear in the polar views.

This grouping according to size corresponds with that of the spermatogonial chromosomes (page 137). These divided themselves into three groups: 10 small, 13 intermediate, and 14 long. Likewise we meet again the same difficulty in drawing the line between the small and intermediate groups.

5. Arrangement on the spindle. The most conspicuous chromosome on the spindle in either polar or lateral views, is often the accessory, as it projects from the edge. This characteristic position is shown in figures 18, 19, 20, 54 to 56, and 58. These drawings and photographs are taken from four different rats. The position was the same in the other animals examined. A small ring chromosome appears close beside the accessory. One of the large chromosomes, but not the largest, usually lies near the accessory, as figures 18, 19, 20, and 55 indicate.

The large ring chromosomes and the accessory group themselves about the periphery. The crosses lie similarly at times, but they are not to be distinguished readily in polar views and

are seldom seen in lateral views. The small and medium-sized chromosomes usually group themselves inside the circle of the large ones.

In passing, it is of interest to note that generally the chromosomes are very closely aggregated, but occasionally one finds a tubule in which they are much more widely spaced. These latter are of course particularly valuable for study. The difference does not seem explicable on the ground of inequality of fixation, as two tubules lying side by side at the edge of a block of tissue will show these opposite conditions.

6. The nucleolus. Two bodies other than chromosomes are found staining like chromatin. These are the nucleolus, or plasmosome, and the chromatoid body, the latter found in the cytoplasm. The nucleolus is early differentiated, being first identified in the pachytene stage (fig. 1.) It stains heavily throughout its history up to late metaphase. By this reaction and its spherical form it can be traced to a late period in the first spermatocyte history. About the period when the chromosomes have formed in the equatorial plate, or just after, it disappears. Its definite form as last seen is shown in figure 27, when the form seems to be spherical with the stainable substance densely aggregated about the periphery. Its size is then less than in its early appearance. I have seen nothing to indicate that it fuses with a chromosome. It does not reappear in the second spermatocyte cells.

The cell from which figure 18 was made shows an irregular body among the chromosomes (*Nu.*), its diffuse character indicated in the drawing by dots. It is stained very faintly by haematoxylin. This body appears in other cells at this stage with the same staining reaction, but is sometimes elongated in form. It may be the nucleolus, which is being absorbed. Its staining reaction does not permit it to be interpreted as a chromosome. This body needs further investigation.

7. History of the accessory. Reference has been made to this chromosome from time to time. Its history is readily outlined. At the time of its earliest positive identification the other chromosomes are in the leptotene stage (fig. 1). It is distinguished

later from the nucleolus by its oval rather than spherical form, and its somewhat lighter staining reaction. In cells of the pachytene stage, it appears as an unevenly bipartite body, the larger part staining more heavily than the smaller usually, whatever nuclear stain may be employed (figs. 1 and 53). Its position close to the nuclear wall, isolated from the other chromosomes by a well-marked space, is another distinguishing characteristic.

During the early tetrad stages of the euchromosomes, the accessory maintains much the character just described. It gradually assumes the form shown in figure 27, a woolly, elongated, curved, rod-like chromosome showing a constriction near the middle. It does not stain quite as densely as the other chromosomes. During this whole period I have not been able to see a longitudinal split. When it appears on the spindle, however, the split is manifest when viewed *en face* or on end (figs. 18, 19, and 54). At this time it is readily distinguishable from the other chromosomes by its rod form, its lighter staining reaction, and its position on the spindle, even when not favorably located or stained for observing its diad nature (figs. 18, 19, 20, 21, 54, 55, 56).

In the first maturation division this chromosome does not divide (figs. 21 and 22). Figure 21 is from a poorly fixed specimen. The view shown in figure 22 was seen many times. Under favorable circumstances this chromosome can be seen in very late anaphase or early telophase maintaining its characteristic position, i.e., extending to one side like a curved finger. Its apparently partial division transversely is a distinguishing mark.

Its failure to divide in the first maturation division produces daughter cells of 18 and 19 chromosomes, respectively, and thus establishes a dimorphism of the spermatozoa.

Cells in interkinesis show frequently a narrow U-shaped chromosome as illustrated in figure 26. This may be the accessory. It should occur in one-half the cells of this stage, but its identification is questionable unless viewed *en face,* as all the chromosomes have assumed the rod form in this stage. It frequently seems to possess the partial transverse division in each

arm which was characteristic of the accessory in the first spermatocyte cells. This U might be interpreted as a V which had failed to bring its arms together again. In that case it would be a euchromosome. If that were the fact one would expect to find more than one in a cell at times. No such observation has been made, so that I am inclined to think it is the accessory and maintains its diad condition in this form through interkinesis. In the euchromosomes the chromatids seem to fuse very closely.

Interkinesis and second spermatocytes

Interkinesis is well marked. The daughter cells of the first spermatocyte division reorganize into a complete nucleus and characteristic cytoplasm, but without spireme formation apparently. In form, the chromosomes, when they are differentiated, resemble the spermatogonial chromosomes in prophase, but in constitution they are very woolly. This condition is shown in figure 25. Later the chromosomes shorten and thicken, the delicate linin threads which have projected from their surfaces lengthen and seem to form bridges between the chromosomes, as seen in figure 26. The process of condensation continues, the woolliness disappears, and the chromosomes group themselves on the spindle as well-defined rods of various sizes. In the larger ones curvature is to be seen. Figure 23 shows a lateral view of three chromosomes and an end view of three others, all just divided. The rod form is maintained in late anaphase, as shown in figure 24 in the case of two chromosomes. The other chromosomes in the figure are also rod-shaped and of unequal lengths, as revealed by focussing. The spindle fiber is attached terminally.

In both figures 23 and 24 only part of the chromosomes present are shown. Counts during any of these stages are difficult on account of the close approximation of the chromosomes. There is, however, no evidence of double reduction, the number of clearly separate bodies being never less than 12 and often as high as 15 in each stage, in addition to some which are obscure.

The spermatids

The chromosomes aggregate about the pole of the daughter cell of the second spermatocyte division and quickly form a mass. This breaks up again into chromosomes which are very woolly and indistinctly differentiated. They gradually become more diffuse, their staining reaction diminishes, and they are lost to view. These conditions are shown in figure 1, the progressive stages, except the final, appearing in the cells marked *Sptd.* 1, *Sptd.* 2, and *Sptd.* 3. I have not been able to make counts nor to distinguish an accessory, the chromosomes are so closely intermingled and interconnected by the woolly threads. Gradual changes transform the spermatid into the mature spermatozoön. Sertoli cells are present and play their customary part. In a few animals studied a small amount of degeneration of these spermatids was seen, but in other animals none. It is doubtful if extensive degeneration is ever the rule in healthy individuals unless at old age, a stage which I have not studied. The cytoplasmic phenomena of special interest are discussed on page 169.

Conclusions with regard to the chromosomes

In the standard albino rat of The Wistar Institute colony the diploid number of chromosomes is 38, the haploid 19; there is one accessory which divides in the second spermatocyte division. These chromosomes are of three forms in the first spermatocytes, viz., the ring (simple or compound), the cross, and the rod, the last-named found only in the accessory. The rings and crosses are tetrad in constitution, so organized that the chromatids may retain their individuality throughout the movements previous to division; fiber attachment is terminal; simple V's are produced in anaphase. The rod form of the spermatogonial chromosomes reappears in interkinesis and in the second spermatocytes, with terminal spindle fiber attachment. The spermatids are dimorphic, arising as they do from dimorphic second spermatocytes of 18 and 19 chromosomes, respectively.

Cytoplasmic structures

A chromatoid body has been mentioned on p. 151. This is a lightly staining mass with chromatin reaction, very small and apparently spherical (figs. 19, 20 and 23). Its first positive appearance is in the late prophase stages of the first spermatocytes after the disappearance of the nuclear membrane. At first it appears to be near the chromosomes, but at later stages it is always found well out in the cytoplasm. In some metaphase cells it is doubled. It is lost during the anaphase of the first spermatocytes and during the interkinesis stage, but reappears in the second spermatocyctes. Nothing of equivalent form is found in the spermatids. In these cells, however, there is a mass lying near the nucleus which stains like chromatin. It develops intensity of staining reaction as the spermatids advance in differentiation. In its fuller development it is seen in figure 1, *Sptd.* 3, where it appears as a globular body, but much larger and staining more deeply than the chromatoid body. In the same figure it is shown less developed in *Sptd.* 1. See page 169 for further discussion.

4. DISCUSSION

The points which need discussion are chiefly the following: the number of chromosomes, the accessory, the constitution of the first spermatocyte chromosomes, the differentiation of spermatogonia and Sertoli cells, the age when spermatozoa are mature, and cytoplasmic phenomena. The first two topics will be considered together.

The number of chromosomes and the accessory

Duesberg ('08) is the first observer to record a chromosome count for the rat. He is uncertain about the number, but places it at probably 24 in the spermatogonia and 12 in the first spermatocytes. It seems strange that he and I should differ so widely in this respect. There is apparently no question about the species of the rat, as we both used Mus norwegicus, var. alb. An accessory is not mentioned by him.

Sobotta and Burchard ('10) state that the number in the egg is 32, but they admit great difficulty in arriving at this conclusion and confess to some uncertainty. They declare with assurance only that there are 8 in each daughter cell of the second division, explaining this number as a second reduction.

Other rodents in which the number of chromosomes have been reported are:

Guinea-pig, Stevens ('11), probably 56; X and Y present.
Rabbit, Bachhuber ('16), probably 22; X and Y present.
Mouse, Sobotta ('95), 24; no mention of accessory.

Of the other mammals for which the number of chromosomes has been reported we have:

Bull, Schoenfeld ('02), 24 or 25, probably 25.
Opossum, Jordan ('12), 18; X present.
Pig, Wodsedalek; ('13), 18; X and Y present.
Horse, Wodsedalek ('14), 37; X present.

The number for man has been given so variously that I have not included the different references in the foregoing list. All one can say at present about it from the literature is that it varies from 12 to 48.

In general a rather high number prevails in mammals. I have examined briefly slides made from guinea-pig and mouse testes and find the large number very manifest, though I have not attempted actual counts. The chromosomes seem better differentiated in the former in each stage and consequently easier of study than in the rat.

The wide variations of the number recorded in man seem due in part to the difficulty of getting fresh material and in part to the unfavorable reaction of the tissue to the traditional fixatives and technique. In view of the recent experiments of Hance ('17), Whiting ('17), and Carothers (MS.) on fixation of insect and mammalian testes, it would seem that the whole problem of mammalian spermatogenesis is to be solved by better technique. The results of these workers agree with my own experience. Good technique gives chromosomes much more clearly separated and consequently more workable than poor technique. It is quite likely that Sobotta's double reduction is apparent

and not real, and that Duesberg's small number and failure to note the accessory are to be explained on this ground. With still better technique than that obtained so far I am quite confident that it will be possible to procure preparations of rat spermatogonia in sufficiently large numbers that many counts may be made, although such difficulties as overlapping and a large mass of chromatin will always remain, as well as the strong tendency of the chromosomes to stay close together in metaphase. Guinea-pig testis fixed by 'B-15' and carried through by the technique which I found best for the rat shows a very promising condition of the chromosomes.

In this connection attention is called to figures 18 to 20. Figure 20 (also 58, a photograph of the same complex) indicates the appearance of the first spermatocyte metaphase plates as seen when the chromosomes are fairly well separated, but either the staining or the fixation has not been delicate enough to differentiate the organization of the chromosomes. The cell from which figure 20 was drawn is from a testis fixed by cold Flemming's fluid to which urea had been added. This fixative, while occasionally isolating the members of the complex pretty well, has never in my preparations, furnished a cell in which the details of chromosome structure could be determined, even when the stain had been extracted to the limit. Figures 18 and 19 are from different rats fixed in 'B-15,' figure 18 from a section, and figure 19 from a smear. The drawings bring out clearly the superiority of this fixation.

Figure 46 is from still another rat fixed in 'B-15.' It shows the excellent general fixation by that method of treatment. In both young and mature testes Flemming's fluid failed in all stages of spermatogenesis to give as workable material in any respect as that fixed in 'B-15.'

Double reduction certainly does not occur widely, if at all, in the albino rat. Perhaps still greater refinement in technique will furnish preparations in which reliable counts can be made of second spermatocyte complexes and their telophases.

An exact correspondence in number of chromosomes in the somatic and spermatogonial cells is not to be expected, in the

light of recent work on other forms. Therefore the number 38 (37 for the male) given in this paper is to be regarded as the basal number about which variations may occur. That a variation is to be expected also in the first spermatocyte chromosomes is doubtful.

The accessory. No worker on the rat has mentioned an accessory. Workers on the mammalia who have recorded the presence of one or more accessories have not analyzed the chromosome organization. Usually the figures bear evidence of poor fixation. In most cases the accessory is determined by either precocity of movement or irregularity of position of one or more chromosomes or by counts. Since the counts are not reliable in poorly fixed tissue, and since both precocity of movement and position are uncertain criteria concerning an accessory, there would remain only the work upon human tissue for discussion. As with the total number of chromosomes, there is also disagreement with regard to one or two accessories. For this reason discussion upon this feature may better be omitted in a paper upon the white rat, especially since none of these observers have attempted to trace the history of their accessory chromosome or chromosomes, and I have no observations of my own to offer upon human tissue.

In this connection it is worthy of note that Von Ebner's figures ('88) for the rat show the accessory and the nucleolus clearly in the early stages of first spermatocyte development, and that Regaud's ('01, '10) do the same.

The striking similarity between the organization of the euchromosomes in the rat and in the Acrididae is found also in the accessory. It is rod-shaped; in the first spermatocytes it isolates itself early, previous to the pachytene stage; throughout early prophase it lies close to the nuclear membrane within a large space; it is characteristically woolly and of lighter staining reaction, and it does not divide in the first division. Further refinement of technique may reveal it in the spermatogonia.

The constitution of the first spermatocyte chromosomes

As brought out in the early part of this paper, the constitution or organization of the chromosomes of the white rat is to be interpreted by regarding them primarily as simple, slightly curved rods with terminal fiber attachment. By the synapsis and longitudinal splitting of homologous chromosomes and by the movement of the parts in the tetrad thus produced, the simple and complex rings and the crosses which appear in the first spermatocyte cells may be explained. Whether the pairing is by telo- or parasynapsis, the fact seems to be that at least one end of the diad is fused, as shown in figure 15. Since there are apparently no unequal chromatids, it is likely that respective pairs of homologous chromosomes are also equal.

While evidence of the tetrad constitution in the diakinesis stage of more of the chromosomes is desirable in order to complete, without question, the morphological history of each member of the complex, it is unnecessary for the large and intermediate-sized ones, as their later stages show this constitution. Comparative evidence from well-established data on chromosome organization is sufficient to fill the gap, since no shape is new and since no peculiarity of organization appears. The field is open for further investigation, to be sure, as the morphological evidence must be the final determinant. Perhaps still more refined methods of technique will give material that will fill this space.

The work on the organization of mammalian chromosomes is very slight and no one has carried it far. Duesberg ('08) shows first spermatocyte figures which indicate the same kind of tetrad formation which I have described, but he does not interpret them as tetrads. In the first division it would appear from his text-figure that he regards them as diads. He is clearly in error in thinking that there is a transverse division of the chromosomes after synapsis by which the various first spermatocyte shapes are produced. Von Winiwarter's figures ('12) for man indicate rings, both simple and compound. Figure 28 shows one directly comparable to the rat chromosome which I have described as *A*. But he does not carry the study on to the organization of the chromosomes.

Neither he nor Duesberg touch upon fiber attachment. This aspect of mitosis is discussed by McClung ('14), who finds that for most of the Acrididae terminal attachment is characteristic. In four genera, however (Stenobothrus, Chorthippus, Chloëaltis, and Trimerotropis), certain chromosomes in the complex have intermediate fiber attachment. The correspondence in fiber attachment between the rat and the Acrididae in general is of much interest because no other instances have been recorded where this mode of attachment is constant in the complex. It will be of interest to learn if it is characteristic of rodents or peculiar to the rat. There is at present no comparative data for any of the mammalia on this point. This persistence of fiber attachment throughout the history of the chromosomes is also another illustration of the close relationship between the tetrad and archoplasmic organization (McClung, '16, p. 675).

The spermatogonia and Sertoli cells

The chief questions of interest here center about the origin and relationships of the spermatogonia and the Sertoli cells, and whether there is more than one kind of spermatogonium. Of associated importance are the number of spermatogonial divisions and the mode of chromosome division.

The first of these problems arises from the presence in the adult tissue of three kinds of cells along the basement membrane. These have been variously interpreted. One kind, the Sertoli cell, was early distinguished and its function interpreted, but its origin is still in dispute. The other two kinds of cell have had at least three distinct interpretations. They are the ones which I have described as type A and type B. Type A was regarded by some as a mother cell of both type B and the Sertoli cells. As such it has received various names, such as spore cell (Brown, '85), *Stammmutterzelle* (Benda, '87), *gonie poussiéreux* (Regaud, '00), and indifferent cell (Schoenfeld, '01).

Regaud's interpretation ('00) of the origin of this indifferent cell in the rat was that it was produced by the amitotic division of the nuclei of the Sertoli cells, which he thought formed a

syncytium that persists into the adult stage. Later ('01) he concludes that the Sertoli cells spring from an early type of cell which he calls 'stem spermatogonia.' These stem spermatogonia have previously fused to form a syncytium which persists in the adult tubule as long as spermatogenesis continues. Neither Schoenfeld (bull) nor Duesberg (rat) agree with this interpretation. Schoenfeld concluded that both the Sertoli cells and the spermatogonia arose from the indifferent cells. Duesberg omits consideration of the Sertoli cells, but regards these indifferent cells as spermatogonia of one type. These divide and produce a second type of spermatogonia (my type B), and these in turn *by division* produce the spermatocytes. This type B cell had been called by Lenhossek ('98) *krustenartige* and by Regaud *croûtelleuses* cells.

In my figures cell type A corresponds with the indifferent or *poussiéreux* (granular) cell, while type B corresponds with the *croûtelleux* (crusty or flaky) cell. It has been noted above that these cells have been regarded as two kinds of spermatogonia. The evidence which I present from the young tissue (figs. 2 to 9), in connection with other evidence which I cite later, leads me to think that there is only one type of spermatogonium, and that this flaky cell (type B) is a first spermatocyte cell in its earliest history, representing the reorganization of the chromatin from the telophase condition of the last spermatogonial division of this cell. Its confusion with spermatogonia may have arisen from the fact that it appears in the adult testis next to the limiting membrane, even to such an extent that in some cross-sections of tubules no other kind of cell can be seen there, and in longitudinal sections a considerable length may be occupied by these cells, frequently to the exclusion of all others. My interpretation of such aggregations of this particular type of cell is that they are the product of a number of actively dividing spermatogonia (type A) in their particular phase of the spermatogenic wave. If successive sections of such regions are examined it is found that Sertoli cells and cell type A are present also in some sections, the latter perhaps in small numbers.

The exceedingly painstaking work of Regaud ('01) on this subject was not accessible to me until after I had come to the conclusion stated in the preceding paragraph. I have gone over my material afresh, but see no reason for changing my original interpretation. Since the question is one upon which I differ from both Regaud and Duesberg, who have done the most upon this subject among the more recent workers on the rat, I detail fully below my evidence and arguments for my conclusion, drawing chiefly upon the series of young rats. Regaud ('01) followed the stages of growth in a series of young rats also, but he bases his argument upon a study of many sections of adult tubules.

The evidence which I have from the young series follows:

1. Type B are the first cells which differentiate from type A in the young tubule after birth.

2. Their first appearance is in very small numbers (fig. 3), but quite general throughout the testis.

3. Cell division is not common in the tubules which show type B abundantly. In rat No. 150, ten days old, some tubules show the outer layer of cells in very active division, but no cells of type B are present. Other tubules show many cells of type B, but no cell division. Occasionally a tubule shows cell division and some cells of type B, but the latter are small and lie side by side, indicating that they are daughter cells freshly produced (fig. 5, *B*). In this last case all the inactive cells are ordinary cells of type A, though some are manifestly preparing for division.

4. Type B cells migrate from the outer edge to the inner portion of the tubule. No cell division is seen in this portion of the tubule (the inner portion) until the first spermatocytes are clearly differentiated by their characteristic mitotic figures.

5. The number of cells of type B usually becomes quite large before these cells advance to the next well-marked stage (leptotene), but the number of these leptotene cells never seems to exceed the number which one would expect from the supply of type B in tubules of the same size.

Argument from the above evidence: The first two points have no particular value alone, since the facts noted might be interpreted to indicate that the cells of type B were differentiated by

growth changes of type A and might divide to produce the spermatocytes. But the conditions noted under items 3, 4, and 5 would not be found were cells of type B spermatogonia, for in that case, 1) cell division would be active in the groups of large undivided cells of that type; 2) these dividing cells would be found in the deeper layers as well as the outer, and, 3) the leptotene cells would be twice as numerous as the cells of type B.

The evidence from the adult tissue corroborates this conclusion, because no cell division occurs there in the layer of cells next the cell membrane after the appearance of type B. Spermatogonial division occurs only once in the wave (except sporadically in the case of a cell or two), and that is at the time of the division of the second spermatocytes and the early growth period of the spermatids. The only cells of type B present then are few and small, and evidently to be interpreted as daughter cells as in figure 5. As the wave progresses, these cells enlarge without division and are crowded into the next inner layer where they pass into the leptotene stage.

Regaud ('01) recognized cells similar to his spermatogonia *croûtelleux* as the early stages of first spermatocytes, and states that the two kinds of cells are likely to be confused. It would seem that his difficulty lay in not recognizing that when cells of type A prepare for division, part of the chromatin becomes at first a flaky, elongated woolly mass which later forms part of the chromosomes, while the remainder of the chromatin gathers in small masses underneath the nuclear membrane and differentiates its share of the chromosomes. His fixation was by potassium bichromate and acetic acid, a fixative which I found produces much distortion. I note considerable difference in the appearance of type B cells according to whether Flemming or 'B-15' is used as fixing fluid. Flemming's fluid produces chromatin masses with hard outlines; 'B-15' with softer, more woolly outlines, yet clear and distinct from each other.

The interpretation of type B cells as the earliest stages of first spermatocytes rather than as a second kind of spermatogonia which must divide to produce spermatocytes, is in agreement with well-known insect material, especially well described by Wenrich ('16) in Phrynotettix magnus.

In this connection Winiwarter ('12) (man) states: ". . . . les noyaux poussiéreux et croûtelleux de la spermatogonèse me semblent représenter des générations successives d'une seule et même catégorie d'éléments: les spermatogonies . . . . ." He does not find them as clearly differentiated as Regaud does in the rat.

As to the number of divisions of the spermatogonia before the final one, much has also been written. I am inclined to think with Schoenfeld that some spermatogonial daughter cells must form spermatogonia as long as spermatogenic activity persists, while others end their career in the differentiation of spermatocytes very early—in fact, some with the production of the earliest spermatocytes. Conditions in the young tubule indicate clearly that at no time are all the cells of type A exhausted, though their relative number decreases enormously from time to time (compare figures 3 and 6). But they increase again actually and relatively during the growth stages of the spermatocytes, as is seen by comparing figure 6 with figure 8, the latter showing many more of type A than the former.

It would appear from these figures, especially figure 5, as well as from conditions found in the adult tubule, that each daughter cell of those spermatogonia which give rise to spermatocytes becomes a spermatocyte—not one a new spermatogonium and one a spermatocyte. This conclusion necessitates that certain spermatogonia (or indifferent cells) are always present in a tubule. These must continue their line by cell division, as brought out in the preceding paragraph. When this supply fails the function of the testis must cease. At what age this cessation occurs has not been determined.[1] The problem thus becomes one of differentiation associated with the wave-like manifestation of energy which finds expression in the complex activities of the spermatogenic tissue—cell division, maturation, growth changes of the spermatids, and the activities of the Sertoli cells correlated with the ripening of the spermatozoa.

[1] While this manuscript was in press, I studied testes from old rats of the Wistar colony, some nearly two years of age—the oldest obtainable. In these spermatogenesis is normal. Rats two years old are likely to die at any time.

I have made only a slight study of the Sertoli cells of the rat, but think there are certain differentiating characters not yet described which appear in the early history of the cells, and which I hope to discuss at length in another paper that will deal more fully with the problem of origin and differentiation. No such distinguishing body as the rod which has been described in man by Montgomery ('11) and Winiwarter ('12), and in the cat by Hague ('16), appears in the cytoplasm of the Sertoli cells of the rat, so far as I can see, nor has any been noted by other workers. In the young testes I find only cells of type A present, and am inclined to think with Winiwarter (man) that the Sertoli cells develop from these, their development being controlled by the factors which control growth.

Regaud ('01) describes degenerating spermatogonia, especially numerous in one stage of the spermatogenic wave. Duesberg ('08) reports that he finds nothing of the kind. I have found in nearly every individual cells such as Regaud describes as degenerating spermatogonia, but they are not confined to any particular phase of the wave. They occur most commonly in rats which are not up to par for some reason not yet fully determined. These cells, as Regaud states and figures, possess a spherical nucleus in which are some chromatic bodies, either spherical or elliptical, of variable size and number. These masses, Regaud states, fuse and gradually disintegrate. Contrary to Regaud, neither Duesberg nor I find any evidence of amitosis.

In concluding this discussion, I shall call attention to the conclusions of Popoff ('09) and Swift ('16) on the development of the sex cells. Popoff finds from his studies in vertebrates that the germinative epithelium early differentiates two kinds of cells, the 'mâle ovule' and the follicle cells. With the lower vertebrates (fishes and amphibians), the male ovules develop into sex cells, the follicle cells into the cells of Sertoli. The male ovules of Scyllium show no signs at all of degeneration. In amphibians and reptiles some male ovules degenerate. With the higher vertebrates (birds and mammals), all the male ovules of the first generations disappear, leaving only one kind of cell, which

produces both spermatogonia and Sertoli cells. These cells, according to Popoff, would in the rat be the ones which I have described as type A. To quote him further:

> L'unité céllulaire resultant de cette dernière transformation n'est toutefois qu'une apparence; les éléments que l'on observe danse cette phase quoique morphologiquement semblables, étant vraisemblablement déjà différenciés: les uns devenant spermatogonies, les autres se transformant en cellules de Sertoli.

Swift studied the chick. His conclusion is: "The primordial germ cells give rise to the spermatogonia, and the coelomic cells of the germinal epithelium produce the supporting cells of the seminiferous tubule."

The mode of division of the spermatogonial chromosomes seemed to me so unmistakably by longitudinal splitting that I was surprised to find in von Winiwarter ('12) the following statement: "J'admets que pour le rat, les chromosomes de la spermatogonie ne possèdent pas une structure suffissament analyzable pour trancher la question (p. 159)," referring to the mode of division. Duesberg ('08) figures the longitudinal division and makes no exception to that as the ordinary mode. I have seen no evidence to indicate another method of division, so that my figure 17 is to be taken as characteristic in this respect. It was not until I read von Winiwarter's statement that I thought a figure showing division of the chromosomes in the spermatogonia would be needed.

The age when the first spermatozoa are ripe

My conclusions on this point differ markedly from those recorded by Hewer ('14). In the white rats studied by her no spermatozoa appear in the lumen until the age of nine weeks after birth, whereas I found them in the Wistar rats at the age of 37 days—26 days earlier.[2] A similar comparative precocity

[2] Some very recent studies on hybrids of gray, dilute, and yellow rats reveal a markedly later onset of differentiation, but I have not yet determined the age when the first crop of spermatozoa appears in either F^1 or later generations. Extracted Albinos of some crosses show much delay.

occurs in the Wistar rats throughout the process of spermatogenesis. Hewer records the first appearance of a lumen in the tubule at seven weeks. My figure 5, which is typical of all the individuals studied of about 14 days of age, shows the lumen present. Hewer's material did not differentiate first spermatocytes from spermatogonia until three and a half weeks after birth. Mine shows this differentiation as far as the pachytene stage at two weeks.

In this connection the following data with regard to the condition of the young rats which I studied will be of interest. With the exception of rats aged 14 and 16 days, the individuals were under weight, as shown in table 1, a fact which would not favor precocity, but rather retardation of development.

TABLE 1

The figures in the first two colums are taken from Donaldson's ('15) tables. The third column is the weight of the rats used in this study, of age corresponding to column 1. The last two columns show the associated cell activities

AGE IN DAYS	BODY WEIGHT IN GRAMS, STANDARD	BODY WEIGHT IN GRAMS, RATS USED IN THIS STUDY	MOST ABUNDANT CELLS	MOST ADVANCED CELLS
10	13.5	12.5	Type A	Pachytene
11	13.9	10.0	Types A and B	Type B
12	14.4	11.7	Type B (slight)	Type B
13	14.9	10.8	Types A and B	Leptotene
14	15.5	23.0	Types A and B	Pachytene
15	16.1	13.6	Types B and C	Pachytene
16	16.7	22.5	Types C and D	Diplotene

The study of this series of young testes shows that the differentiation of the spermatocytes from the indifferent cells of type A (figs. 2 to 15) is a process which, in the early stages, involves all the tubules of the testis, and to an extent very nearly equal in each tubule, although at any given age in days the stage reached may vary somewhat. The change from this generally similar condition of all the tubules to that of the differential activity in parts of the tubule characteristic of the adult testis is a gradual one.

In the early stages the degree of variation in individuals of nearly the same age is not great; e.g., in rat no. 150, 10 days old,

weighing 12.5 grams, very few tubules showed any stages beyond type A. Some showed type B, while those having advanced as far as the pachytene stage were very few indeed, and are to be regarded as exceptional.

Rat No. 149, 11 days old, weighing 10 grams, exhibits no pachytene stages, but a considerable development of type B cell. The 12-day rat, weighing 11.7 grams, showed very little development of type B, these few cells being found in only a few tubules. In this case the whole organ seemed to be retarded. The 13-day rat, weighing 10.8 grams, shows type B cells in great abundance throughout the organ, and in a few tubules a few cells in the leptotene stage. The tissue of the 14-day rat, No. 146, weighing 23 grams, is much more advanced than the 13-day specimen. Many of the cells are in the leptotene and a few have reached the pachytene stage. Type B is in great abundance. No tubule shows only type A. The whole organ exhibits the growth stages. While cell division is perhaps not very vigorous, cells of type B are intermingled with type A in some tubules, and in some cases the cells of the latter type have been crowded inward by type B, as shown in figure 5, so that type B occupies all the outer layer of cells next to the limiting membrane. This condition indicates that just previous to the death of the rat, cell division of type A had been very active, but had ceased with the production of this outer layer of daughter cells, which by the time the rat was killed had advanced to type B stage.

The 15-day rat, weighing 13.6 grams, shows about the same degree of development as the 14-day specimen, except that the cells in the more advanced stages are less numerous. Cell division, however, is very abundant. Various parts of the testis were examined, revealing a uniform condition. The 16-day rat, weighing 22.5 grams, shows in general a slight advance over the 15-day rat, the most developed cells being in the diplotene condition, the next step in advance of growth over that reached in the 15-day testis.

From this stage on the development of the cells is slower. Great complexity of cell types has been reached and the whole organ is growing rapidly. The weight of the testes at age 37 days

is 0.244 grams, as compared with 0.067 at 14 days (Donaldson, '15). The weight of the organ from the time when the pachytene cells are well developed until the first crop of spermatozoa is produced is thus almost quadrupled.

I have found in the literature no other account of the age at which the various steps in the process of spermatogenesis occur.

The age of 37 days for the first crop of spermatozoa correlates closely with the time of descent of the testes, which occurs "about the fortieth day of age or somewhat earlier" (Donaldson, '15).

Cytoplasmic structures

Two cytoplasmic bodies were noted by the early observers of mammalian germ cells. These have been variously designated, but to-day they go by the names of idiosome (figs. 1, 25 and 26) and chromatoid body (figs. 1, 18 and 20). According to Lenhossek ('98), the idiosome was first described in 1847 by Merkel in the guinea-pig; the chromatoid body by von Brunn in 1876. The idiosome was later noted by other observers in different mammals, and now is regularly expected to be found in all.

A complete recapitulation of the literature on these bodies is unnecessary, but I will outline the views of the chief or more recent workers. The most complete discussion is to be found in Lenhossek ('98) and Duesberg ('08).

The problems which have arisen in connection with the idiosome are three: its origin, its connection with the centrosomes, and its fate. Lenhossek found it appearing earliest in some spermatogonia, but not well differentiated there, and figures it containing two small bodies that stain more deeply, which he calls the centrosomes. I have not been able to find the idiosome present in the true spermatogonia. Lenhossek's figure is not of a spermatogonium, as I interpret the cells, but of a young first spermatocyte in the stage which I have indicated by cell-type B. In fact, all of Lenhossek's figures of spermatogonia are of this cell-type B. I have searched both old and young material for some evidence of this body in spermatogonia, but find none.

Nor is it present in the young material in even the pachytene stage, although in the adult tubule it is plainly visible in this stage (fig. 1, *P.c.*). Its absence in the young material may be due to the fact that the cytosome is not yet fully differentiated when the pachytene stage is reached.

With regard to the nature of the body, Lenhossek objects to identifying it with the nebenkern of the invertebrates on the ground that the germ cells of mammals have no corresponding body. He objects also to classifying it as an attractive sphere, or as archiplasma, since in cell division it does not divide, and each part accompanies a centrosome to its pole, nor does it take part in the formation of the spindle. He says the centrosomes are naked at the poles of the spindle, and that the idiosome (he calls it the *Sphäre*), remains beside the equatorial plate during the formation of the spindle.

> Wider alles Erwarten . . . lässt sich noch in ungetheiltem Zustande als ein etwas geschrumpfter, noch immer lebhaft färbbarer Körper seitlich neben der Spindel während der ganzen Phase der Spindelbildung und auch der Aequatorialplatte; erst mit dem Beginn der Metakinese entzieht sie sich dem Blicke, offenbar durch Auflösung und gleichmässige Vertheilung ihrer Substanz auf das ganze Cytoplasma der sich theilenden Zelle; muss sich doch ihre Substanz in gleicher Menge in den beiden Theilungshälften der Zelle finden, da sie sich doch in den Tochterzellen zu je einer neuen Sphäre zu construiren hat (pp. 243 and 244).

According to this author, the behavior of the idiosome is the same in both spermatocytes. In the spermatids he thinks its function is to take part in forming the acrosome.

With two exceptions, my observations agree with Lenhossek on the behavior of this body as far as to the spermatids, after which I have not followed its history. The first exception has already been noted above, viz., that it is not found in the spermatogonia. The second one is in regard to the staining reaction. I do not find that the body takes the stain more deeply when the chromosomes lie in the equatorial plate, with either Flemming or 'B-15' fixation, or any stain, and am inclined to think that he confused with this body a chromosome which is late in taking its place, as his figure shows an irregular body instead of a spherical

one, a body which I have identified as a tardy chromosome—usually chromosome A.

Duesberg agrees with me that the idiosome is not found in the spermatogonia, but makes its appearance in the early stages of spermatocyte growth. He does not describe its division, but shows the centrosomes within it. He agrees with Lenhossek that it disappears without dividing in each of the spermatocyte divisions, but differs from him in stating that its staining reaction weakens as the cell prepares for division. In the spermatids he decides that a part helps in the formation of the acrosome and the other part is lost in the cytoplasm which is eliminated.

Von Winiwarter ('12) notes it first in the growth stages of the first spermatocytes, but in this respect he states that he differs from both Branca and Montgomery (man) and Schoenfeld ('01) (bull). As to the division of the idiosome, he states that "it disappears and perhaps degenerates" when the first spermatocyte divides. He does not mention it, nor do his figures show it in the later stages. He states that the central corpuscles remain within the idiosome until the pre-equatorial plate is formed, when they emerge and separate to form the spindle. Montgomery ('12) (man) states that he finds "no trace of a sphere in spermatocytes nor spermatids."

The chromatoid body was also early noted. Lenhossek ('98) treats it at length, and identifies it with the large characteristic body in the cytoplasm of the spermatid to which I have referred (p. 155) and figured (fig. 1). He thinks its fate is to take part in a temporary structure connected with the spermatozoon head. Montgomery ('12) does not mention it nor figure it. Von Winiwarter ('12) does not find its exact equivalent, but notes "a certain number of chromatophiles" which may conjugate into "a sort of little rod." These chromatophilic corpuscles occur in more or less abundance during all the stages of maturation, he states, and notes a 'chromatoid body' in the spermatids in addition to the chromatophile corpuscles.

Duesberg ('08) (rat) identifies the large heavily staining body in the cytoplasm of the spermatids as the chromatoid body, traces it briefly in connection with the centrioles (without stating

a function) until it lies against the nuclear membrane at the time when the centrioles have placed themselves at the same point, after which it breaks up into three small bodies which gradually disappear in the cytoplasm after moving away from the nucleus. As I have not studied spermiogenesis, I am in no position to criticise these manifestly opposite results of Lenhossek and Duesberg.

5. CONCLUSIONS

1. The diploid number of chromosomes in the male albino rat (Mus norwegicus, var. alb.) is 37; the haploid number is 19.

2. Sperm dimorphism is produced by the presence of one unpaired accessory. It divides in the second maturation division.

3. The constitution of the first spermatocyte chromosomes is typically tetrad, with the four parts so organized that each may retain its individuality.

4. The shapes of the chromosomes in the spermatogonia are all curved rods; in the first spermatocytes, simple and compound rings, crosses, and one rod, the accessory; in the second spermatocytes, curved rods.

5. Fiber attachment is terminal in the univalent chromosomes throughout, and is fixed.

6. No synizesis has been observed.

7. Only one type of spermatogonia is present. These develop from apparently indifferent cells which produce the Sertoli cells also.

8. The first spermatocyte cells begin to differentiate between the seventh and tenth days after birth.

9. The first spermatozoa are ripe between the thirty-sixth and fortieth days after birth, about the time of the descent of the testes.

10. A nucleolus (or plasmosome) is present in the first spermatocytes. It seems to disintegrate and does not reappear in the second spermatocytes.

11. The specialized cytoplasmic structures are the idiosome and the chromatoid body.

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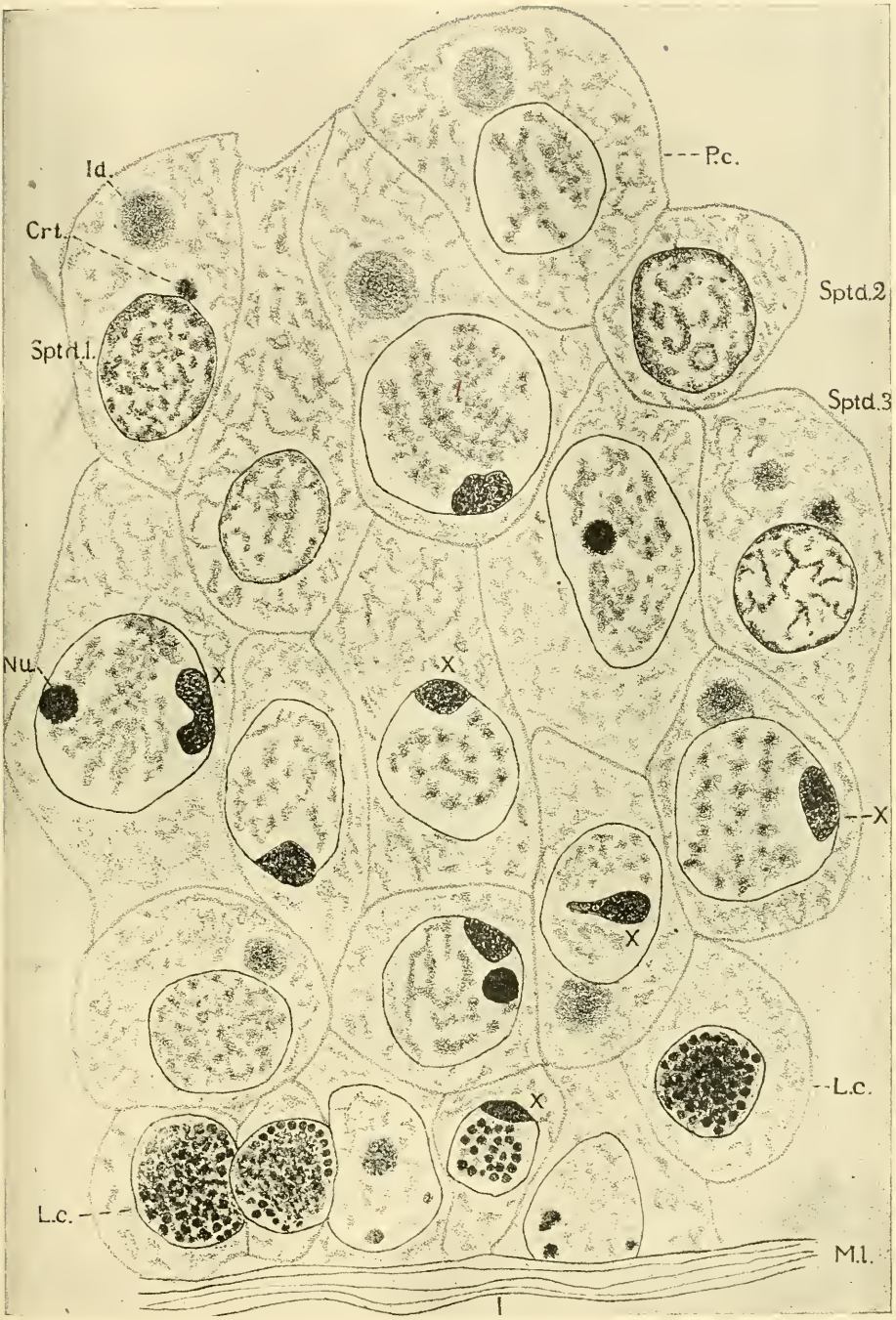
EXPLANATION OF PLATES

The drawings were made with a camera lucida at an original magnification of 2800 diameters, except figure 1, which was at 880 diameters. They were then enlarged by projection and worked over for details. In reproduction they have been reduced to the magnification indicated. The photo-micrographs were made with a Bausch & Lomb fluorite system 1.9-mm. oil-immersion objective, N. A. 1.32, Zeiss 2 or 4 projection ocular, and Watson holoscopic oil-immersion condenser, with the exception of figure 46, which was made with a Zeiss 4-mm. objective, the other lenses as above. The photographs have been reproduced at the original magnifications. The unit of magnification is 1400 diameters except for Plate 2 and fig. 46. Fixation was by 'B–15' unless otherwise specified.

PLATE 1

EXPLANATION OF FIGURES

1 Portion of wall of tubule of 50-day rat. *Crt.*, chromatoid body; *Id.*, idiosome; *M. l.*, membrana limitans; *Nu.*, nucleolus; *P.c.*, pachytene cell; *Sptd.*, spermatid; *X*, accessory; *L.c.*, leptotene cell. × 2100.


Id.
Crt.
Sptd.1.
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L.c.
L.c.
M.l.

PLATE 2

EXPLANATION OF FIGURES

2 to 8 show changes in the germinative wall and the size of the tubule in rats from 7 to 14 days after birth. All × 800.

9 to 14 show steps in the transformation of the undifferentiated cells to the pachytene stage of development. All × 1200.

Abbreviations: *D.C.*, dividing cell; *A.B.C.*, cells of certain types.

2 Oblique section of tubule from 7-day testis. Section 10 micra thick; stained with iron-haematoxylin and orange G. Germinative cells all alike (type *A*). Two dividing cells (*D.C.*) appear, one out of focus.

3 Transverse section of tubule from 11-day testis. Section 2 micra thick; stained with iron-haematoxylin and acid fuchsin. Differentiation has begun, as shown in cell type *B*.

4 Section of tubule from 12-day testis. Section 2 micra thick; stained with iron-haematoxylin and acid fuchsin. Differentiation has progressed to cell type *C*.

5 Portion of transverse section of tubule from 14-day testis. Section 5 micra thick; stained with iron-haematoxylin. Shows dividing cells (*D.C.*) of type *A* organizing into cells of type *B*; also three cells in pachytene stage.

6, 7 and 8 from the same testis as figure 5; thickness and stain also the same. Figure 6 is from a slightly oblique section; figure 8 from one as nearly transverse as could be found. The outer row of cells in figure 6 is chiefly of type *B*, the result of the process taking place in figure 5; the next row passing toward the lumen shows cells chiefly of type *A*; three cells in the pachytene stage are seen as in figure 5. In figure 7, upper portion, cells of type *C* have replaced those of type *B*, figure 6, and have crowded inward to lie between cells of type *A*. Compare figures 9 and 10. The lower tubule in figure 7 and figure 8 show progressively later stages.

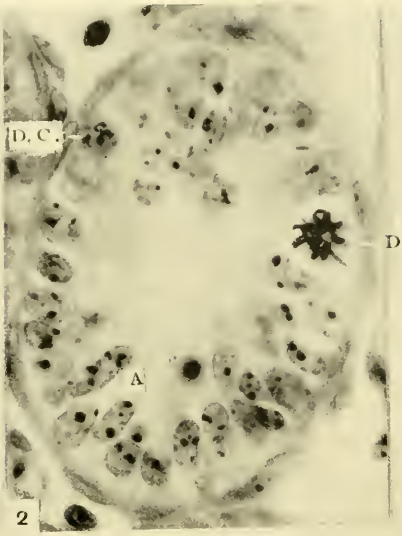
9 to 12 are from 15-day testis; 13 and 14 from 16-day. All from sections 5 micra thick and stained with iron-haematoxylin.

9 and 10 show details of types *B* and *C*; compare with figures 3 to 7.

11, 12 and 13 show the progressive development of the spireme up to its leptotene stage. Compare with figure 7, lower tubule, and figure 8.

14 illustrates the pachytene, or paired thread stage.

15 is a sketch made from the same section as figure 14; magnification not taken.



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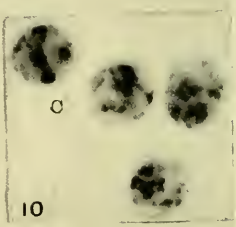

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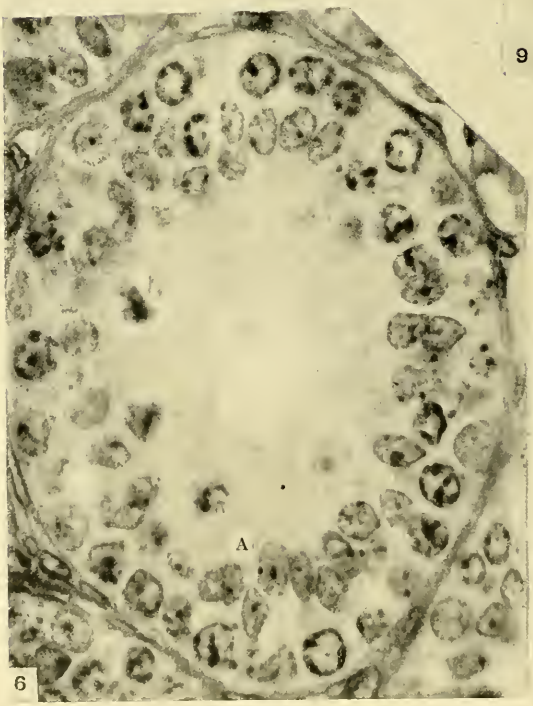

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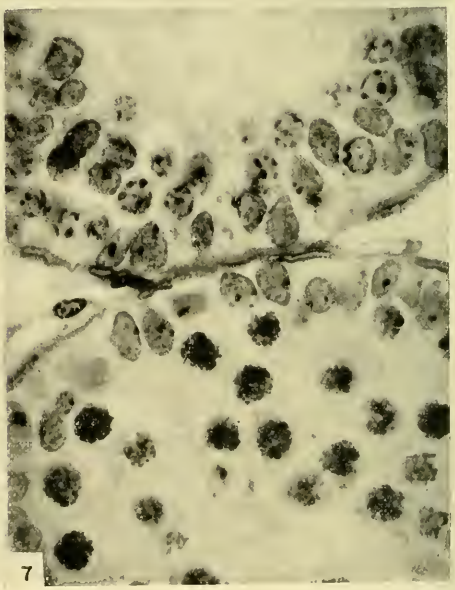
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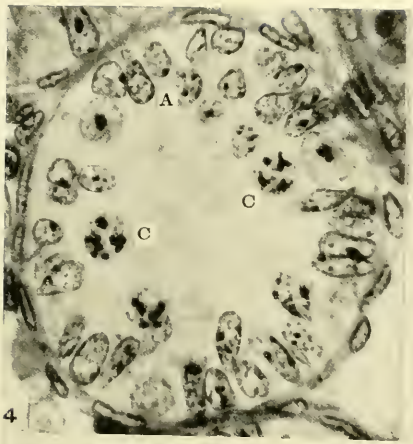
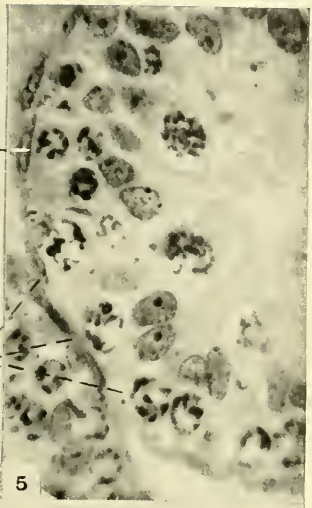

A
C
C
4


B
D.C.
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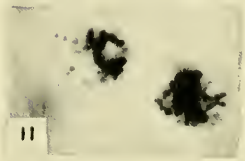
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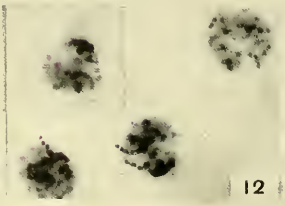
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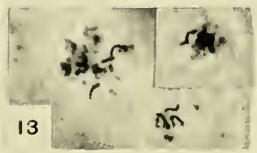
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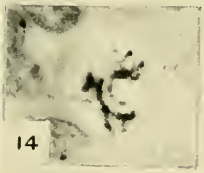
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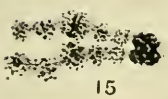
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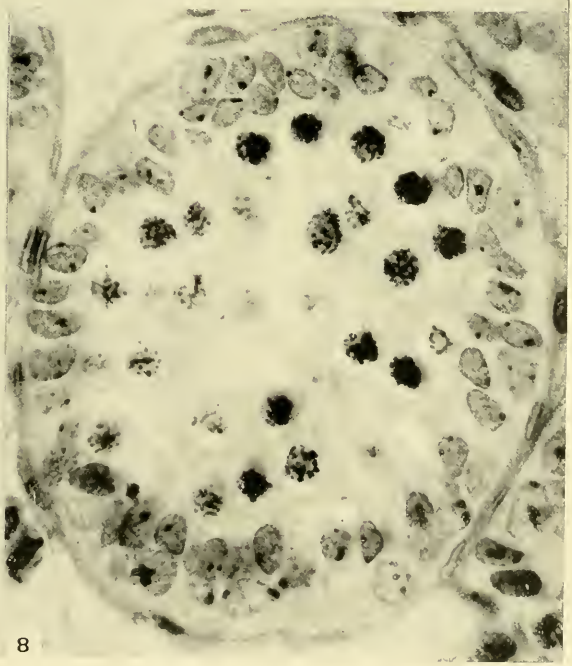
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PLATE 3

EXPLANATION OF FIGURES

16 Spermatogonial complex in metaphase. *A*, *A*, the largest pair of chromosomes. This figure represents the full number in the complex—37. From a 7-day old rat. × 4200.

17 Division of spermatogonial chromosomes. Only a few of the chromosomes are shown, some in lateral and some at end view. From same rat as figure 16. × 4200.

18 to 20 Polar views of first spermatocyte complexes. The letters, *A*, *B*, *C*, *D* designate particular chromosomes; *X*, the accessory; *Crt.*, the chromatoid body; *Nu.*, nucleolus or plasmosome. Chromosomes drawn with dotted lines in figure 18 are those whose detailed structure could not be determined. *C(?)* is either a ring or a cross, probably a ring. In figure 19, the large, irregular chromosome in the center, just above *D*, seems to be a cross. Figure 19 is from a smear; the other two from sections. Figure 20 is from a testis fixed in Flemming's fluid. × 4200.

21 Early anaphase from a poorly fixed first spermatocyte (the only figure taken from this animal). The chromatin is badly clumped, but the different divisions determinable are indicated. It is introduced to show the accessory passing undivided in the first spermatocyte division. × 4200.

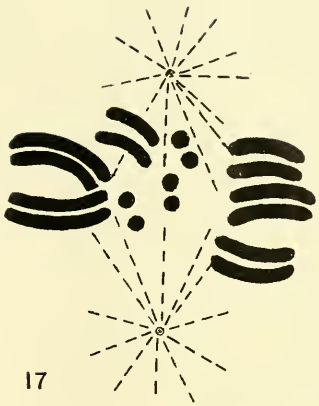
22 Late anaphase from a well-fixed first spermatocyte. Accessory (*X*) at only one pole. One large chromosome appears not yet fully divided; some simple V's are also evident. × 4200.

23 Second spermatocyte; some of the chromosomes in early anaphase. × 4200.

24 Second spermatocyte; some of the chromosomes in late anaphase. × 4200.

25 and 26 Two stages in interkinesis. Figures in each case show only a part of the total number of chromosomes present. × 4200.

16



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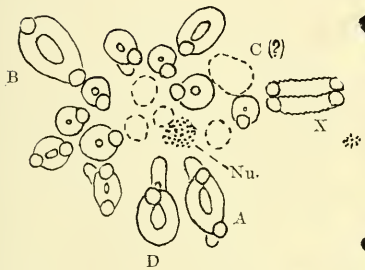

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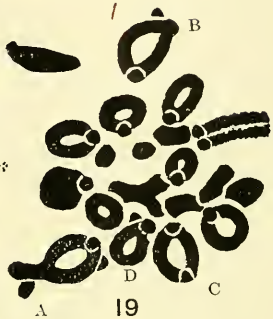

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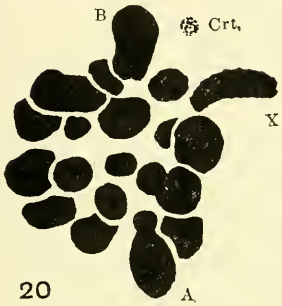

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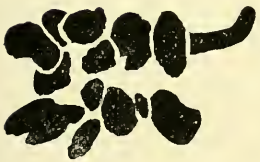

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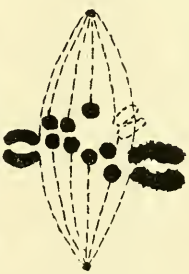

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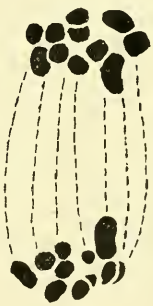

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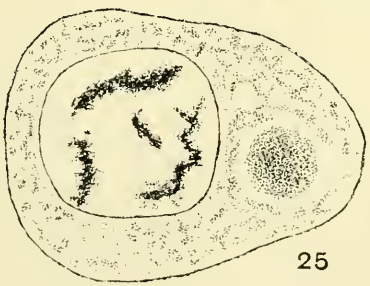

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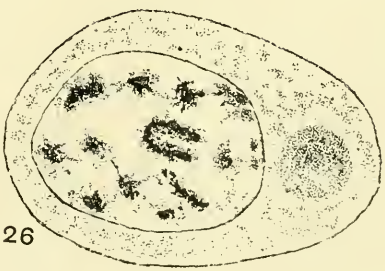

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PLATE 4

EXPLANATION OF FIGURES

Studies on the organization of the first spermatocyte chromosomes. The figures are all at a magnification of 5600 diameters.

27 to 31 show the various forms of chromosomes present in the albino rat.

27 Eighteen chromosomes drawn from one cell. *A,B,C,D,* particular chromosomes as in figures 18 and 19; *Cr.*, cross; *Nu.*, nucleolus; *R*, ring; *X*, accessory.

28 A chromosome not seen in the cell from which figure 27 was made, but found in a neighboring cell.

29 A cross in midprophase (in a somewhat earlier stage than that of the chromosomes of figure 27).

30 to 33 Prominent stages in the history of chromosome *A* when it forms as a triple ring; the subsequent stages are essentially similar to those shown in figures 38 to 42. The organization of this form is interpreted diagrammatically in figure 43.

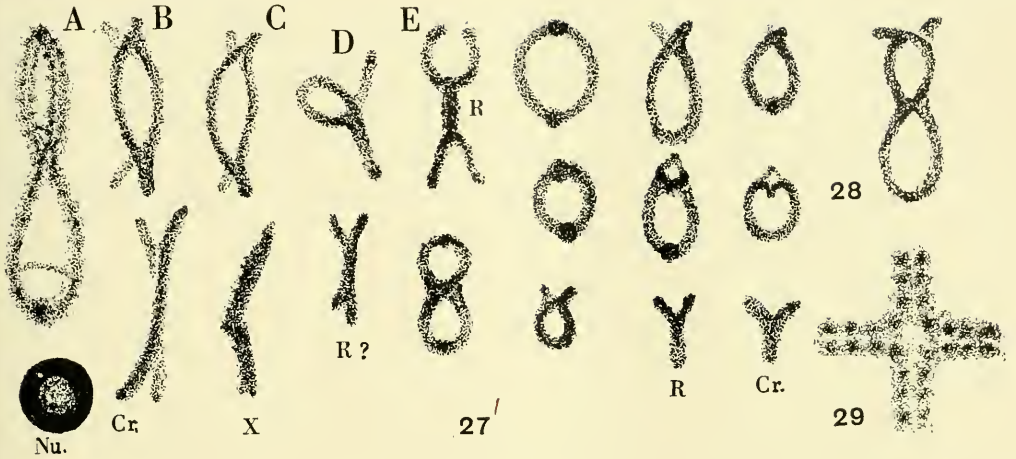
34 to 42 show the history of a large chromosome when it forms as a double instead of a triple ring.

36 A side view of figure 35.

37 A similar view at a slightly later period; the chromatin is more condensed.

41 and 42 show the formation of the simple V's in anaphase.

43 to 45 Drawings from McClung ('14) (original from Granata), showing my interpretation of the organization of the simple and complex ring chromosomes.


A
B
C
D
E
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R
Cr.
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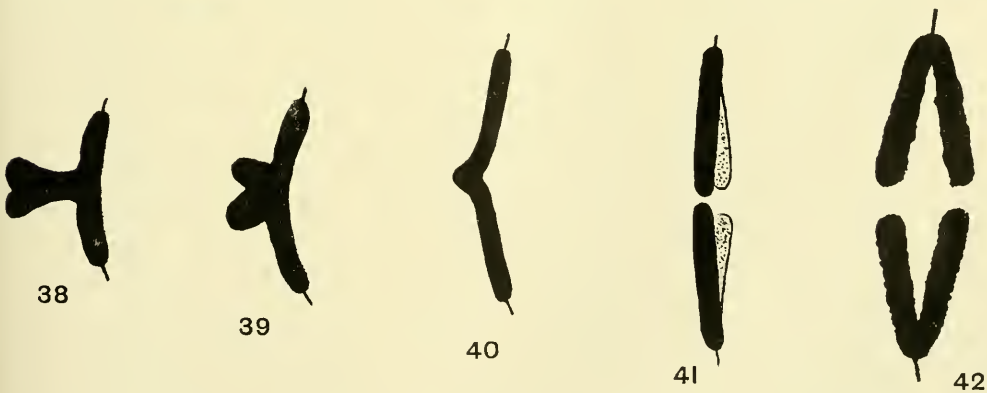
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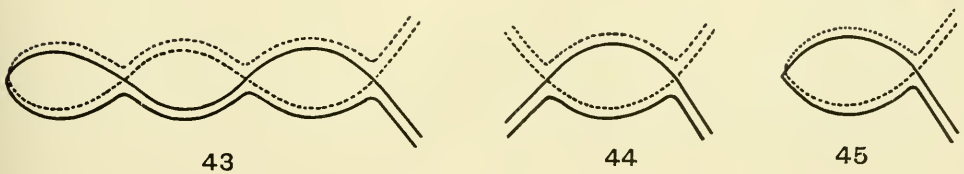
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PLATE 5

EXPLANATION OF FIGURES

46 Portion of tubule of 50-day rat. The tubule is cut nearly parallel with the length, but to one side of the center, so that the lumen barely shows. First spermatocyte prophases and metaphases are the most prominent cells. Simple rings appear in cells just above the figure number. The darkly staining cells close to the lamina propria are in the leptotene stage (toward the right); the cells showing finely dotted nuclear contents in the same zone (to the left) are in the pachytene stage. Compare figures 7 and 8. Spermatogonia are few and at the stage shown in this figure are not dividing. Iron-haematoxylin. × 400.

47 to 58 Single chromosome and complexes. Iron-haematoxylin. × 1400.

47 shows in upper cell chromosome *A* in diakinesis.

48 shows the character of the filaments in early diakinesis. Compare with the lower cell in figure 47.

49 Chromosome *B*. This photograph has been retouched to show the full chromosome, as its outline follows the nuclear wall so closely that the whole chromosome would not focus at one level with the oil-immersion lens.

50 The small double ring. Compare with figure 27.

51 A ring chromosome with widespread lugs. This chromosome is drawn in figure 35.

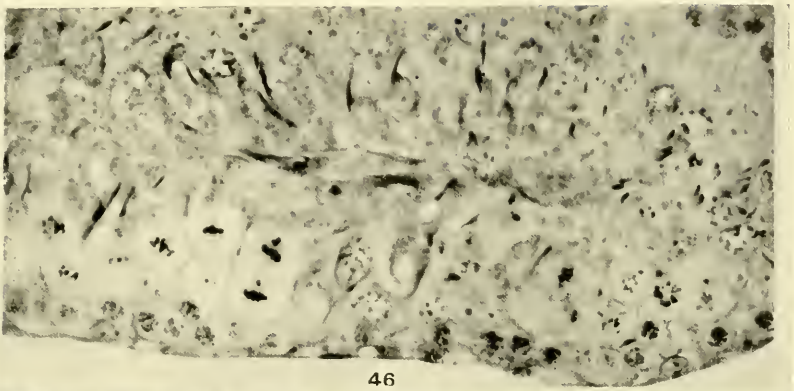
52 One of the large crosses in prophase.

53 shows the accessory when the other chromosomes are in synapsis, one of which appears at the left.

54 to 56 The characteristic position and staining reaction of the accessory in first spermatocyte metaphase and early anaphase.

57 A cross in metaphase; lateral view. The neighboring chromosomes have been obliterated in order to bring it out clearly.

58 A polar view of the first spermatocyte complex in metaphase. This has not been retouched. The same cell is drawn in figure 20. Flemming fixation.



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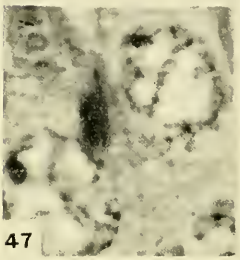

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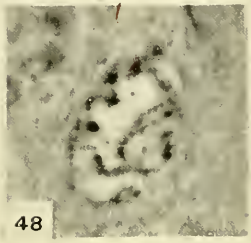

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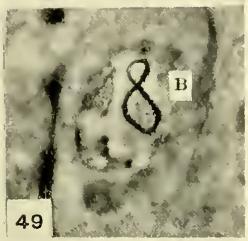

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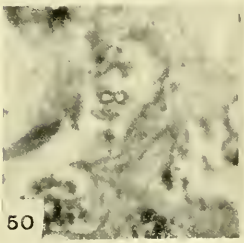

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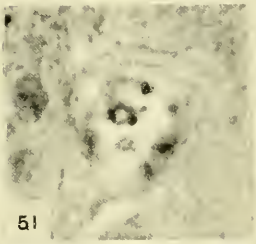

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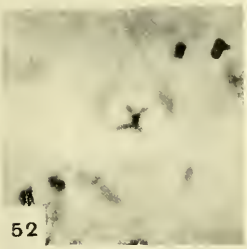

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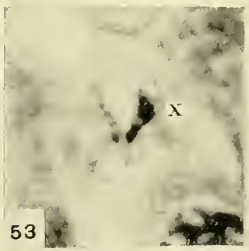

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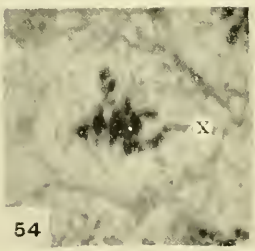

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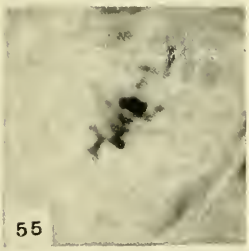

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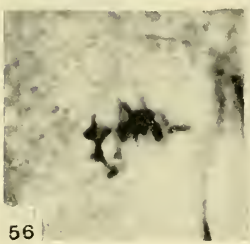

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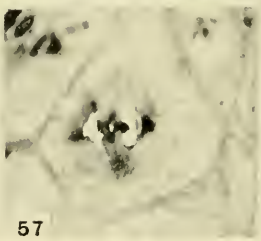

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE MORPHOGENESIS OF THE THYREOID GLAND IN SQUALUS ACANTHIAS

E. H. NORRIS

From the Institute of Anatomy, University of Minnesota

TWENTY-THREE FIGURES

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1. INTRODUCTION

The morphogenesis of the elasmobranch thyreoid presents many problems of more than intrinsic interest and import, because of their bearing upon the large number of researches regarding the development and structure of this organ throughout the vertebrate series. The thyreoid gland of this form shows with almost diagrammatic clearness, in its earlier stages, a number of features which are rendered relatively obscure in forms which are more complex or undergo a more rapid development. The gland further exhibits a number of characteristics, both during its early development and during the follicular period, which are of particular interest in the light of recent work on the human thyreoid gland (Norris, '16). Moreover, the organ presents a very fortunate field for a continued study of the relation of parenchymatous and vascular structures.

This study was undertaken in the Anatomical Laboratory of the University of Minnesota at the suggestion of Prof. R. E. Scammon, under whose supervision the work was conducted.

I wish to thank Dr. Scammon for his valuable aid and criticism and for the loan of material from his embryological collection.

2. MATERIAL AND METHODS

The present paper, based upon an extensive series of embryos of Squalus acanthias, gives an account of the development of the thyreoid from the time of its earliest appearance until the adult structure is attained.

The following table, in which the embryos are arranged in the order of their lengths, shows the specimens used in this work. A large part of the material is from the Harvard Embryological Collection (H.E.C.), while some is from the Embryological Collections of the Department of Anatomy of the University of Minnesota (M.E.C.), the personal collection of Dr. R. E. Scammon (S.C.), and the collection of the Department of Zoology of the University of Kansas (K.U.E.C.). The material marked M.E.C., as well as the older new-born specimens, were obtained by Dr. Scammon from the Harpswell Biological Laboratory. My thanks are due for the loan of material. An asterisk (*) following the collection number signifies that the cervical region of the embryo alone was sectioned. Otherwise the entire embryo was available in serial sections.

The ordinary reconstruction methods, both plastic (Born's waxplate method) and graphic, were utilized in the present study. In all cases where a determination of the follicular form or structure was attempted, special precautions were observed in making the reconstructions as accurate as possible.

The drawings for reconstruction were made with the camera lucida or with Edinger's projection apparatus on transparent paper. After the drawings were completed, those of successive sections were superimposed upon a tracing-table, and the corresponding structures in each section were then given a letter or number. The drawings were controlled throughout by careful microscopic observations, to determine the frequently complicated relations of neighboring follicles. By this method it was possible to determine with certainty the limits of any particular mass or follicle.

SERIAL NUMBER	COLLECTION NUMBER	LENGTH IN MILLIMETERS	SECTION THICKNESS IN MICRONS	CORRELATION OF MATERIAL[1] WITH	
				Normal-plate numbers	Balfour's stages
1	M. E. C. 685	2.0	7	7	D, E, F
2	M. E. C. 686	3.0	7	10	G
3	H. E. C. 1498	3.8	8	17	H
4	M. E. C. 679	4.0	7	16	
5	K. U. E. C. 449	5.0	10	20	
6	M. E. C. 614	5.0	7	20	
7	H. E. C. 1335	5.2	6	20	
8	S. C. 17	5.4	10	19	
9	K. U. E. C. 545	5.8	10	19	
10	H. E. C. 1497	5.8	8	19	I
11	H. E. C. 1821	6.0	10	19	
12	M. E. C. 612	6.0	7	21	
13	S. C. 29	6.3	10	21	
14	S. C. 15	7.5	10	22	
15	S. C. 24	7.5	10	22	
16	M. E. C. 615	7.5	7	22	
17	S. C. 12	7.5	10	22	
18	H. E. C. 1495	9.0	8	23	
19	S. C. 20	10.0	10	23	
20	S. C. 16	10.1	10	23	K
21	S. C. 50	11.5	20	24	
22	M. E. C. 645	11.6	7	24	
23	S. C. 18	13.3	10	25	L
24	S. C. 3	14.0	10	26	
25	M. E. C. 613	15.0	7	26	
26	S. C. 1	15.5	10	26	
27	S. C. 52	18.0	10	27	
28	S. C. 4	19.0	10	27	
29	M. E. C. 646	19.0	8	27	
30	S. C. 2	19.0	10	27	M
31	M. E. C. 647	19.8	8	27	
32	S. C. 5	20.5	10	27	
33	H. E. C. 1494	20.6	10	28	N
34	H. E. C. 1493	21.0	10	28	
35	S. C. 53	22.0	10	28	
36	M. E. C. 629	24.0	10	29	
37	S. C. 6	28.0	10	30	
38	H. E. C. 1357	28.0	10	30	O
39	S. C. 25	33.0	12	31	
40	M. E. C. 680	33.0	10	31	
41	S. C. 8	33.1	12	31	
42	S. C. 9	36.0	12	32	
43	S. C. 7	36.0	12	32	

SERIAL NUMBER	COLLECTION NUMBER	LENGTH IN MILLIMETERS	SECTION THICKNESS IN MICRONS	CORRELATION OF MATERIAL[1] WITH	
				Normal-plate numbers	Balfour's stages
44	S. C. 10	36.5	10	32	
45	S. C. 11	48.0	12		
46	S. C. 54	48.0	12		
47	H. E. C. 427	60.0	14		
48	S. C. 55	80.0	12		
49	H. E. C. 1882	95.0	12		
50	New-born 2*	160.0	10		
51	New-born 5*	160.0	10		
52	New-born 1*	170.0	10		
53	New-born 6*	200.0	10		
54	New-born 3*	210.0	10		
55	New-born 4*	ca.250.0	7		

[1] In correlating embryos of the present series with Normal-plate numbers and with Balfour's stages only the general development of the embryo has been considered and not the state of development of the thyreoid gland.

3. PREFOLLICULAR PERIOD

a. Organogenesis

The anlage of the thyreoid gland in Squalus acanthias makes its appearance in embryos of approximately 4 mm. in length as a solid epithelial bud from the floor of the pharynx. This bud, which is at first little more than a localized thickening of the entodermal lining of the pharynx, is placed just ventral to the point at which the oesophagus leads from the pharynx and in the region inferior and caudal to the ventral extremities of the first two pairs of gill pouches (figs. 1, 2, 17). A study of these figures will show the absence of any groove or pouch in the floor of the pharynx at the point where the thyreoid first appears. Not only is there an entire absence of a groove or pouch, but, as shown in figures 1 and 2, the anlage even protrudes somewhat into the cavity of the pharynx. The bud increases in size and, extending caudally, assumes a pedunculated form, being suspended from the floor of the pharynx by a rather extensive but very narrow neck. This condition obtains in embryos of 5.8 mm. (No. 9 of present series), where the gland mass and the neck by which it is suspend-

ed are nearly coextensive. By the time the embryo has reached 14 mm. in length the thyreoid is better described as keel-shaped (fig. 18). The gland mass and the suspending neck are no longer coextensive, for the former has grown caudally until it is nearly three times as long as the latter. By the time the embryo has attained a length of 19 mm. the gland has severed its connection with the pharynx and has the form of a column with rounded ends and whose cross-section is ovoid (fig. 19). But the gland does not long preserve its columnar form. By the time the embryo has reached a length of 24 mm. the gland is well advanced in its transition, being neither columnar, as was the condition at 19 mm., nor completely flattened, as in the succeeding stages. This transitional stage is well set forth in figure 20. At 28 mm. the

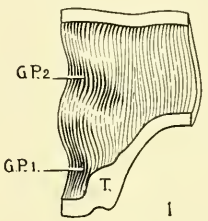

Fig. 1 Graphic reconstruction of the pharynx of an Acanthias embryo 3.8 mm. long (No. 3). *G.P.*1, and *G.P.*2, first and second gill pouches, respectively; *T.*, thyreoid. × 75.

gland has quite lost its columnar form of the earlier periods and has assumed the form of a flat, diamond-shaped plate whose surfaces are quite smooth and regular (fig. 21).

Between 28 and 33 mm. the gland again changes in form, and only at this stage (33 mm.) can it be said of the organ for the first time that has in miniature the form of the adult thyreoid (fig. 22). For descriptive purposes the gland is readily divisible into two parts, an anterior, the corpus, and a posterior, the cauda. The corpus, as seen from above or below, is rhomboidal in outline, its transverse diagonal being a little greater than its anteroposterior; while the cauda, on the other hand, presents the form of a long straight bar which joins directly with the posterior angle of the corpus in front and terminates posteriorly in a pointed extremity. The body is nearly three times as thick as the tail, but the

two parts are quite alike as regards regularity of outline and smoothness of surface. The ventral surface of the tail is grooved along nearly its whole extent by a closely applied blood-vessel.

In embryos between 33 mm. and 50 mm. in length (which latter approximately marks the end of the prefollicular period), the gland continues its growth and increases considerably in size. But notwithstanding the increasing mass of the organ, it constantly maintains through succeeding stages an outline which simulates that of the stage last described.

One further observation on the external form should be noted. Almost without exception, the glands, subsequent to the period in

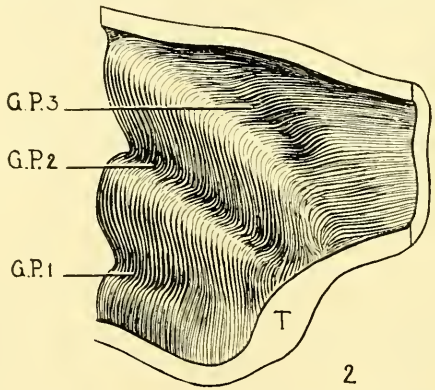

Fig. 2. Graphic reconstruction of the pharynx of an Acanthias embryo 5 mm. long (No. 6). *G.P.*1, *G.P.*2, and *G.P.*3, first, second, and third gill pouches, respectively; *T.*, thyreoid. × 75.

which they sever their connection with the pharynx, present a small mound-like projection from the dorsal aspect of the body (corpus). This little mound is quite regularly located in the central region of this surface.

b. Histogenesis

The modifications in the external form of the gland are accompanied by, and to some extent at least, dependant upon a number of internal changes.

The anlage of the thyreoid gland when studied in cross-section appears as a solid diverticulum or bud from the floor of the

pharynx. The cells which make up this bud are quite like those of the entoderm from which it is derived. The cell outlines are not apparent. The thyreoid nuclei, which are all very similar, contain one, two or three large masses of chromatin, which are round or oval in shape and smooth in outline, except for one or two small chromatin threads which may extend outward from each mass. These chromatin masses are generally closely applied to the nuclear membrane. The remainder of the nucleus is filled with a clear nuclear sap through which run a few delicate and faintly staining fibrils. This type of nucleus, which has been described and figured by McGill ('10), Neal ('14), and Scammon ('15), is characteristic of the young Acanthias embryo, being found also in the cells of the mesenchyma, mesothelium, medullary canal, in the hepatic anlage, and elsewhere. In the earliest stages, all of the nuclei are placed with their long axes perpendicular to the surface of the thyreoid bud.

This condition is present only in the early anlage while the gland mass is diminutive. As soon as the gland increases in size (embryo of 5 mm.), changes are noticeable both in the structure and arrangement of the nuclei. The chromatin masses above mentioned become more irregular and there are given off from them a number of chromatin strands which eventually form a coarse network. The chromatin masses may also be somewhat broken up, being separated from the nuclear wall so as to present a granular appearance. But the change in form of the nuclei is perhaps even more striking than their altered structure. In place of the elongated ovoidal nuclei of the early anlage, which were in all respects like the nuclei typical of the general entoderm, the gland at this stage presents nuclei which appear in the cross-sections to be nearly spheroidal. These nuclei have diameters which are approximately the same as the short diameter of the nuclei found in the general entoderm. Those which are most ovoidal lie in the peripheral part of the gland and are placed with their long axes perpendicular to the surface. The nuclei of the central part of the gland, on the other hand, are very irregularly disposed. At this same stage (embryo of 5 mm.) a large number of vacuole-like spaces can be seen which are quite generally dis-

tributed throughout the gland mass. Whether these are intra- or intercellular spaces cannot be said, for the cell boundaries are not visible. The margins of these spaces are extremely irregular and indistinct, blending almost imperceptibly with the faintly staining cytoplasm. These changes are shown in figure 3.

In embryos of 5.8 mm. the structure of the gland is again changed. As pointed out in the preceding section, the thyreoid of

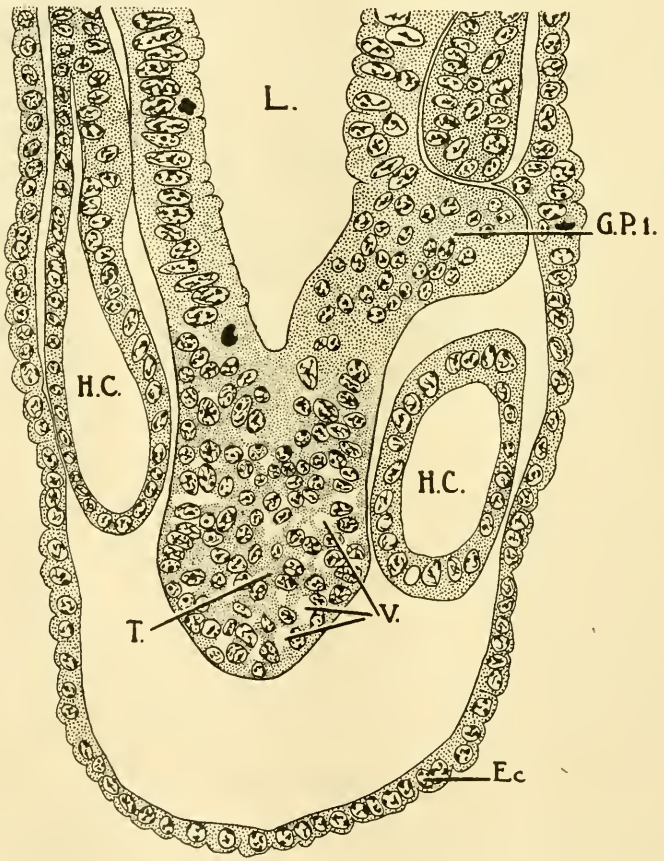

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Fig. 3 Drawing of a part of a cross-section of an Acanthias embryo 5 mm. long (No. 6). *Ec.*, ectoderm; *G.P.*1, first gill pouch; *H.C.*, head cavity; *L.*, lumen of pharynx; *T.*, thyreoid; *V.*, vacuole-like spaces. × 300.

this stage has the form of a pedunculated bud suspended from the floor of the pharynx by a very narrow neck. Figure 4 is a cross-section of the gland at this stage and shows the pedunculated form as well as the structure of the bud. No cell boundaries are apparent in this region, either in the entoderm lining the pharynx or in the glandular epithelium. The nuclei are quite characteristic in form, arrangement, and structure. As regards form and arrangement, two general types of nuclei may be noted; the first, those which are elongately ovoidal and which are found in a cir-

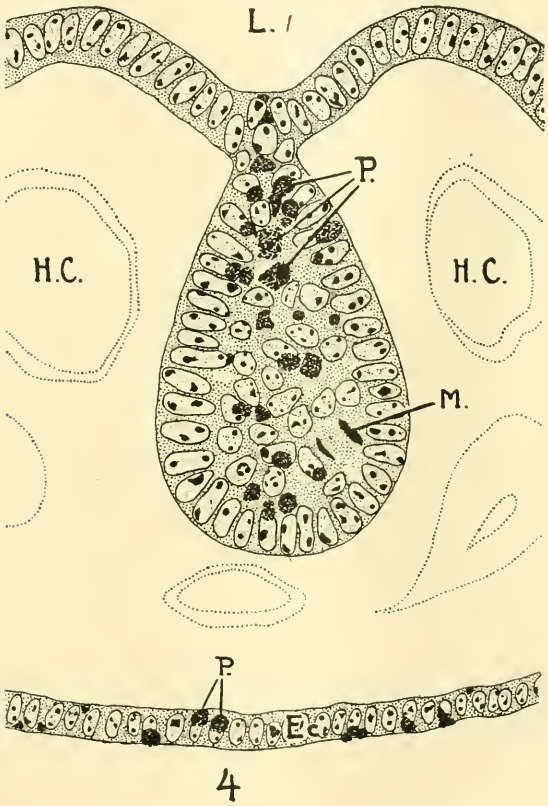

Fig. 4 Drawing of a cross-section through the thyreoid gland and adjacent structures of an Acanthias embryo 5.8 mm. long (No. 9). *Ec.*, ectoderm; *H.C.*, head cavities (outlined in stipple); *L.*, lumen of pharynx; *M.*, mitotic figure; *P.*, pigment granules. × 500.

cumferentially disposed row around the periphery of the thyreoid bud, as well as in the cells lining the pharynx. The second type, those which are spheroidal or at most slightly ovoidal, are found both in the center of the thyreoid bud and in its suspending stalk. Structurally, these two types of nuclei are very similar. They possess a distinct nuclear membrane and regularly contain two large, smooth, chromatin masses which do not lie in apposition with the nuclear wall. These chromatin blocks are suspended in a clear nuclear sap through which run a few faintly staining linin fibrils. Certain vacuole-like spaces, similar to those described in the gland of a 5-mm. embryo, are also present in this stage and may be noted in the succeeding members of the series up to approximately 20 mm.

As shown in figure 4, the gland at this stage (embryo of 5.8 mm.) contains a large amount of pigment which is scattered throughout the gland mass, but is accumulated in largest quantities in the neck of the bud. This pigment is made up of highly refractive, yellowish-brown granules which are closely packed together in masses or blocks of variable size. Whether this pigment is intra- or intercellular cannot be determined with definiteness. Certain of the blocks seem to be superimposed upon underlying nuclei, while others appear to be lodged in the cellular interspaces. A similar pigment is found in the ectoderm of this same region (fig. 4). This pigmentation of the gland occurs in all the specimens from 5.8 mm. up to about 19 mm., at which time the gland severs its connection with the pharynx. Thereafter the pigment decreases rapidly, a little being present even up to 24 mm.

Histologic evidence of the rapid growth of the gland during these early stages is seen in the large numbers of mitotic figures found in nearly every section. In a single longitudinal section through the gland of a 21 mm. embryo, ten cells in karyokinesis were counted.

At 19 mm. there appear within the gland a number of completely closed cavities. These cavities are at first only tiny clefts, appearing very much as though the cells around them had only pulled apart a little in their formation. These small clefts

soon increase in size and are thus brought more closely into relation with one another. Nothing more can be said by way of generalization regarding their size, shape, or arrangement, great differences being found in these respects both in cavities of the same gland as well as in glands in different stages of development. These intraglandular cavities are not at all like the vacuole-like spaces described above. In every case these cavities, unlike the vacuole-like spaces, are outlined by a very distinct and sharp margin, and in no case is there any visible content within the cavity. It is important to note that these spaces are quite independent of any external conditions, and in no case in these early stages do they open to the outside of the gland. Furthermore,

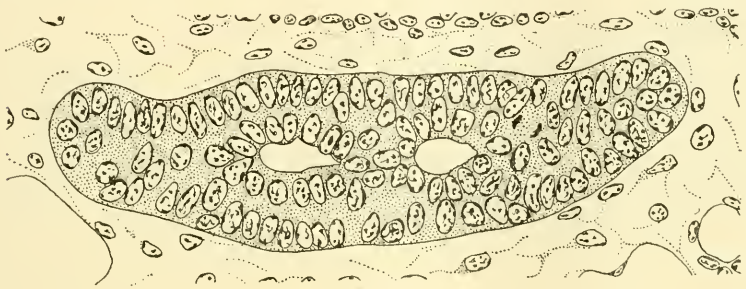

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Fig. 5 Drawing of a cross-section through the thyreoid gland of an Acanthias embryo 28 mm. long (No. 37). × 450.

as shown in figures 5, 19, 20, 21, 22, they develop independently of one another as isolated spaces and only secondarily may become confluent. At a much later stage (28 mm.) these cavities, for the first time, open to the outside and are invaded by blood-vessels. These cavities develop only in the body of the gland and have never been observed in the region of the tail (cauda).

Careful examination of sections of the gland in this period of its development shows that this process of cavity formation is accompanied or paralleled by—probably even preceded by—four other processes, in all of which the cells actively participate. The first of these is the beginning differentiation of cell boundaries, which originally are very faint and indistinct, but which

are well formed and easily seen some time before the cavities have opened to the outside.

The second process consists of a change in form of the nuclei. The centrally located spheroidal nuclei become ovoidal like those occurring in the periphery.

The third process is very closely allied with the second and has to do with the rearrangement of the nuclei. The peripherally disposed nuclei maintain the position they have constantly held, while those of the central part of the gland which have become ovoidal, instead of remaining irregularly scattered, now assume a definite relation to the developing cavities. As a result of their rearrangement, their long axes are placed perpendicular to the margins of the cavities. By this process the vast majority of the nuclei in the thyreoid establish a definite relation to some glandular surface, whether external or internal.

The fourth process, that of cell proliferation, is, as already mentioned, remarkably rapid during this period.

As a result of these processes, the gland is changed from the form of a solid epithelial column into a number of epithelial plates. These plates, which are two cells in thickness, possess surfaces which are quite smooth and which are very intimately related to the blood vascular system. It is from these plates that the primary follicles develop.

4. FOLLICULAR PERIOD

A thyreoid follicle may be defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells. As pointed out in a previous paper on the morphogenesis of the follicles in the human thyroid gland (Norris, '16), this definition includes all the features of the follicle which may be regarded as absolute and constant. The size and shape of the follicle may vary, and great differences are found in these respects in follicles of the same gland as well as in follicles of embryos at different stages. Typically the thyreoid follicle may be considered spherical or spheroidal in shape; but, as will appear later, this type is subject to considerable variation. The term primary

follicle will be used to include those follicles developing independently of pre-existing follicles. The follicles derived from pre-existing follicles, by budding or otherwise, are termed secondary follicles.

In the present series, the first primary thyreoid follicles appear in an embryo 60 mm. long (No. 47). In this specimen the thyreoid gland has essentially the same structure as has that of the last stage described in the prefollicular period, i.e., it is made up chiefly of longitudinally placed epithelial plates or bands, which are only two cells in thickness. But in this stage, the plates, which have in previous stages been characterized by com-

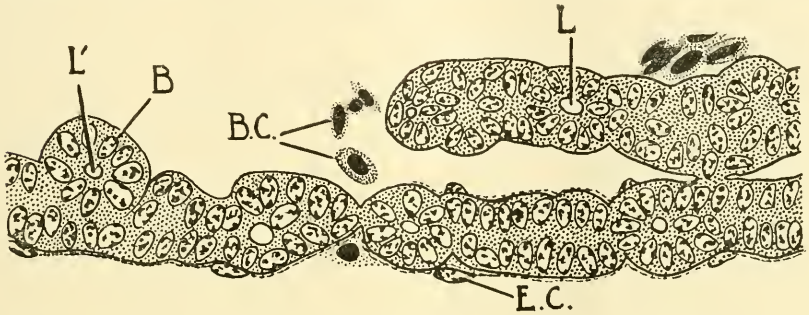

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Fig. 6 Small portion of a cross-section of the thyreoid gland in an Acanthias embryo 160 mm. long (No. 50), magnified to show cell structure and the relation of the developing primary follicles to the epithelial plates. Note the appearance of lumina (*L*) in buds (*B*) from the surface of the plate as well as in swellings along its course (*L'*). *B.C.*, blood corpuscle; *E.C.*, endothelial cell. × 450.

paratively smooth surfaces, now present surfaces which are more or less roughened by the appearance of scattered hillocks or mounds (figs. 6, 23). They are placed very irregularly with respect to one another, and may appear for the first time in any part of a plate, at its periphery or in a more central region.

Cross-sections (fig. 6) show that these hillocks are the immediate anlagen of the early thyreoid follicles. Further, the hillocks are apparently produced by the concurrence of four different processes in the epithelium. The first process is that of cell rearrangement, the second that of cell proliferation, the third that

of absolute cell growth, and the fourth that of lumen formation. These processes, although described separately, may occur simultaneously.

The first departure from the two-celled plate arrangement, in the process of follicle formation, is found in a rearrangement of the cells of the plate (fig. 6). The cell outlines can be made out only with difficulty in most cases. But in those places where they can be seen, they bound cells which are more or less columnar in form. The nuclei are ovoidal or elliptical in outline and are placed with their long axes perpendicular to the surface of the plate. Here and there along the course of the plate (fig. 6) some of the nuclei have shifted their axes and have changed their relative positions. Certain of the nuclei have rotated through an arc of 90 degrees so that their long axes, in their final position, are at right angles to their original position in the plate. As a result of this shifting process, little circlets (really spheres) of nuclei are formed in the plate.

This shifting of the nuclei is but the visible expression of the changing position of the cell. For while it is impossible to observe the cell boundaries in most cases, it is hardly probable that the nuclei shift their axes independently of the cytoplasm; moreover, the few faint cell-boundaries which may be made out show the same changes in position as do the nuclei. Further, it is usually found that at the point from which a nucleus has shifted toward the center of the plate a slight depression appears on the surface of the plate, indicating that the cytoplasm has shared equally with the nucleus in this movement. From these observations it may be concluded that the first process manifested in follicle formation is the shifting of the axes of certain cells of the epithelial plate through an arc of 90 degrees.

This process results in the transformation of the smooth surfaces of the bands (fenestrated plates) into surfaces which are somewhat roughened. Apparently the irregularities are not due, at first, to swellings on the plates, but rather to the slight indentations produced by the shifting of certain cells toward the center of the plate as above described. In cross-sections (figs. 6, 13, 14) such a plate appears as a sort of beaded chain, with

alternate swellings and constrictions. But, as noted above, the initial swellings due to this process are only apparent and actually are not greater in thickness than is the plate in other parts of its extent where indentations have not yet occurred.

The extraordinary cellular activity of the epithelium at this stage is clearly manifested by the large number of mitotic figures. The localization of these mitoses is even more significant than is their number. The nuclear figures are usually found only in those places in the epithelial plates where actual thickenings on the plates are being formed. Therefore the little mounds which appear on the plates, as the immediate anlagen of the early follicles, may be formed not only by the rearrangement of the already existing cells of the epithelial plates, but also by the formation of new cells as well. Consequently, it can easily be seen how the apparent swellings on the plates, produced by the rearrangement of the existing cells, may be transformed into actual swellings by the absolute increase in number of the cells found in a localized area. These swellings become roughly spheroidal in form.

The third process referred to above is the absolute increase in size of the cells. While the cells are shifting their axes nd proliferating, they are also increasing in size. This progressive increase in the height of the cells corresponds in general to the progressive stages in the differentiation of the two-celled plate into newly formed follicles. So that the thyreoid gland of an embryo 60 mm. long presents, in different regions, epithelial cells varying greatly in height. The lowest cells are found, at the beginning of the process, in the two-celled plate; the highest being found at the other extreme, in the completely formed follicle.

Three of the four processes above mentioned as apparently involved in the evolution of the follicle from the epithelial plate have now been reviewed in detail. The formation of the lumen remains to be considered. Just preceding the appearance of the lumen, the spherule (in which it is about to develop) appears in cross-section as a circlet of columnar cells, whose nuclei are peripherally placed. This arrangement results in the formation of a striking picture. The nuclei are regularly placed at the

periphery of the circle and form a dark band, which surrounds a clear, central cytoplasmic portion. The magnitude of this cytoplasmic area and the sharp contrast between the two portions (in the stained preparations) are usually prominent features. The lumen makes its appearance in the center of this cytoplasmic area as a tiny spherical space outlined by a definite and regular margin. It is as though the cells had but drawn apart a little, so that their central ends, instead of remaining in contact with one another, are separated by an interval. It is important to note that no tubular stage is found in the process of lumen formation, but, as was pointed out in the case of the human thyreoid gland (Norris, '16), the lumina appear as absolutely independent spaces.

When the lumina first appear they have no apparent content; but undoubtedly they contain some substance which is not stained with the ordinary method and which increases in amount with the size of the follicular cavity. Certain of the larger lumina (not all of them), which are perhaps older, are found to contain a hazy, granular substance. Typical colloid does not appear until later, in the 160-mm. stage (No. 50).

In the foregoing account, the cell masses in which the lumina develop have been described as spherules whose cross-section is circular in outline. While this is true for typical follicles, some variation, within comparatively narrow limits, is found. Ovoidal or somewhat irregular follicles occur, but these are not more numerous than would be expected in a rapidly growing tissue.

These stages, which have just been described, may be summarized very briefly. The comparatively smooth epithelial plates of the prefollicular period have been transformed into plates with rough surfaces. The roughenings on the plates are the early indication of the follicles about to be formed. With the progressively increasing number of follicles the plates are transformed into irregular bands, which in turn give rise to groups of solid or hollow masses of cells.

Although the first well-formed primary follicles are found in embryos about 60 mm. long, the epithelial plates of the prefollicular period are only very gradually transformed and re-

placed by follicles derived from them. Primary follicles continue to be formed until the embryo attains a length of approximately 250 mm. Apparently, by this time, the epithelial plates have been completely used up in the formation of follicles.

At 250 mm. the thyreoid is made up of follicles which are variable both in size and form. Apparently the follicles tend to assume a spheroidal form, but through crowding and mutual pressure of the adjacent elements, they are forced to assume ovoidal or irregularly angular shapes (fig. 7). The follicle wall consists of a single layer of low columnar epithelial cells which show evidences of secretory activity. Colloid, which begins to appear at about 160 mm. is abundantly present in the 250 mm. stage.

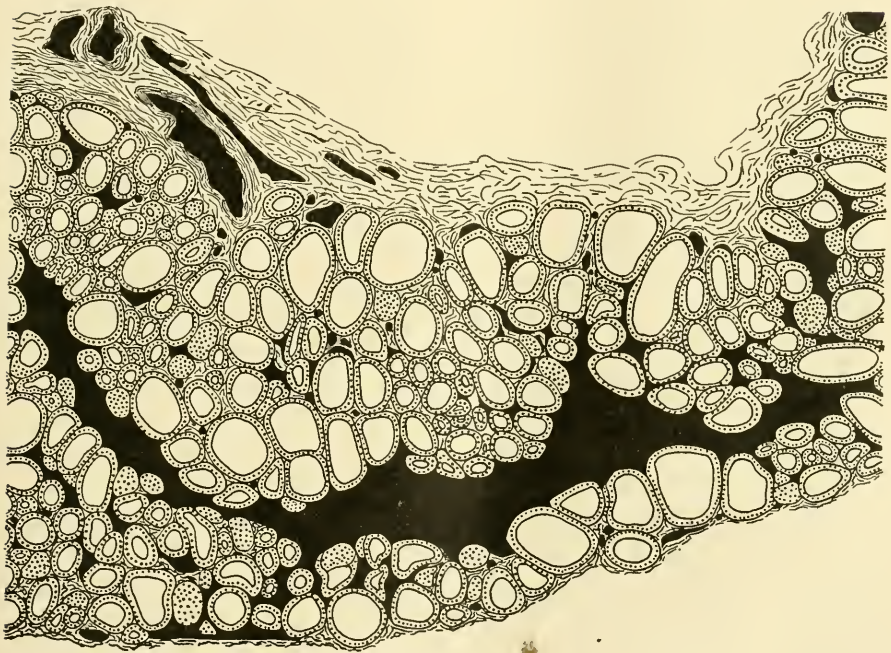

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Fig. 7 Semidiagrammatic drawing of a cross-section through the thyreoid gland of an Acanthias embryo 250 mm. long (No. 55), showing the relation of the vascular system to the parenchymatous elements of the thyreoid. The colloid which was present in nearly all of the follicles has not been shown in the drawing. × 40.

Up to and including the 250 mm. stage, no evidences of the formation of secondary follicles have been observed. The material available from stages later than those included in the table of embryos studied was not suitable for histologic study. However, the thyreoids from three adult specimens were obtained and, although the glands were poorly preserved, certain observations were possible. A number of irregular follicles with bud-like processes are present which suggest the formation of secondary follicles.

5. BLOOD SUPPLY

From the time of its first appearance the thyreoid gland in Squalus acanthias is associated more or less intimately with the vascular system. In the early embryos, in which the gland is just recognizable, the ventral aorta bifurcates immediately caudal to the gland, so that its two most anterior branches (first arches) pass dorsally on either side of the thyreoid. These thin-walled vessels lie in immediate apposition with the thyreoid anlage (fig. 8), and although they give off no branches to the gland, they probably suffice to nourish all the tissues of this region.

Soon after these vessels have associated themselves with the thyreoid, mesenchyma begins to invade the region and to grow around both the vessels and the gland, so that by the time the embryo has reached a length of 5.8 mm. (No. 645) the mesenchyma, through its rapid growth, has formed a dense tissue which separates the blood-vessels from the gland for a considerable distance (fig. 9). This relation between the gland and the vascular system is maintained until the time at which the gland is cut off from the pharynx (ca. 19 mm.). At this stage another set of vessels begin to invade the region in which the thyreoid is placed. These are thin-walled venules growing forward from a trunk which buds from the common cardinal vein just before it opens into the sinus venosus. This venous trunk (external jugular or linguofacial vein) buds from the common cardinal in embryos of about 14 mm. in length. It grows downward along the side of the pericardium and comes to lie ventral to the heart and to the thyreoid. It breaks up into a wide-meshed network

of sinusoidal venules around the latter (figs. 4, 15). This condition is found in embryos of about 19 mm. in length.

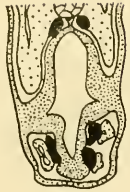
In an embryo of 20.6 mm. (No. 33) this venous plexus establishes the first apparent communication with the arterial system, through anastomoses which it forms with small twigs derived from the mandibular branch of the first aortic arch. The association of the thyreoid with the arterial system is never very extensive and the arterial blood which the gland receives is small.

The subsequent changes in the vascular system in its relation to the thyreoid gland are found to be: first, a more intimate association of vascular and parenchymatous elements from stage to stage; second, a marked and progressive increase in the area of the vascular bed locally; third, a very evident tendency of the venules around the thyroid to fuse and by their confluence to form the large 'thyroid sinus' (Ferguson, '11). These changes are clearly portrayed in the series of figures 10 to 15.

Apparently the blood-vessels grow around the thyreoid gland and spread out in the spaces between its plates without exerting any appreciable mechanical influence on it. The gland is, as it were, passively enveloped by the coalescence of the vessels around it. Although this is true in the early stages, during the latter part of the follicular period the follicles increase in size so as to considerably decrease the relative expanse of the thyreoid sinus. By their growth the lumen of the thyreoid sinus is encroached upon, one main drainage channel of considerable size being left, and a large number of capillary sinusoids (Minot, '00) are formed (fig. 7).

The vessels appear to have no causal relationship to the formation of the follicles, as the follicles do not begin to appear until after the sinus is well formed. Moreover, as noted above, the follicles of the Selachian thyreoid develop in the same manner as those of the man, and in this latter form there is no such relation between the glandular elements and the blood-vessels.

The endothelium lining the sinusoids forms a more or less complete investment for the epithelial portions of the gland. In most places the endothelium is present, but in some parts the epithelium appears to be in immediate contact with the blood stream.



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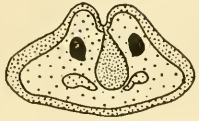

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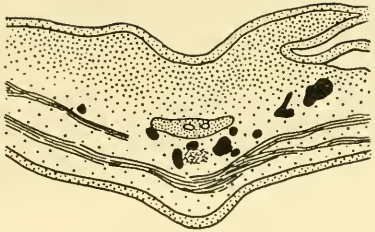

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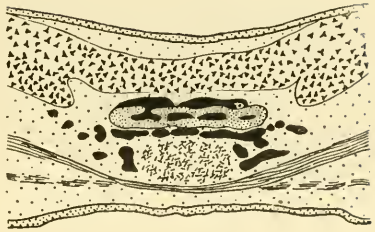

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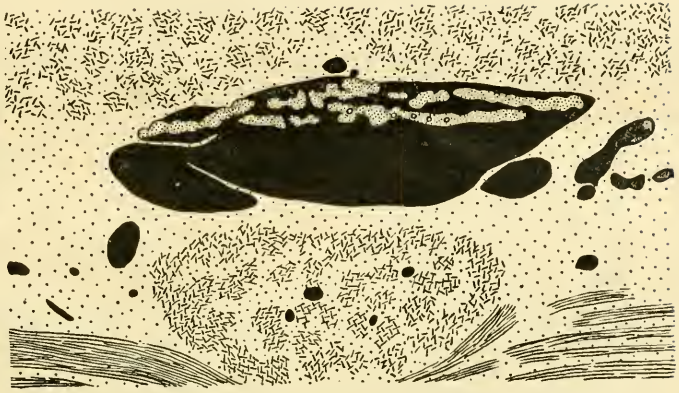

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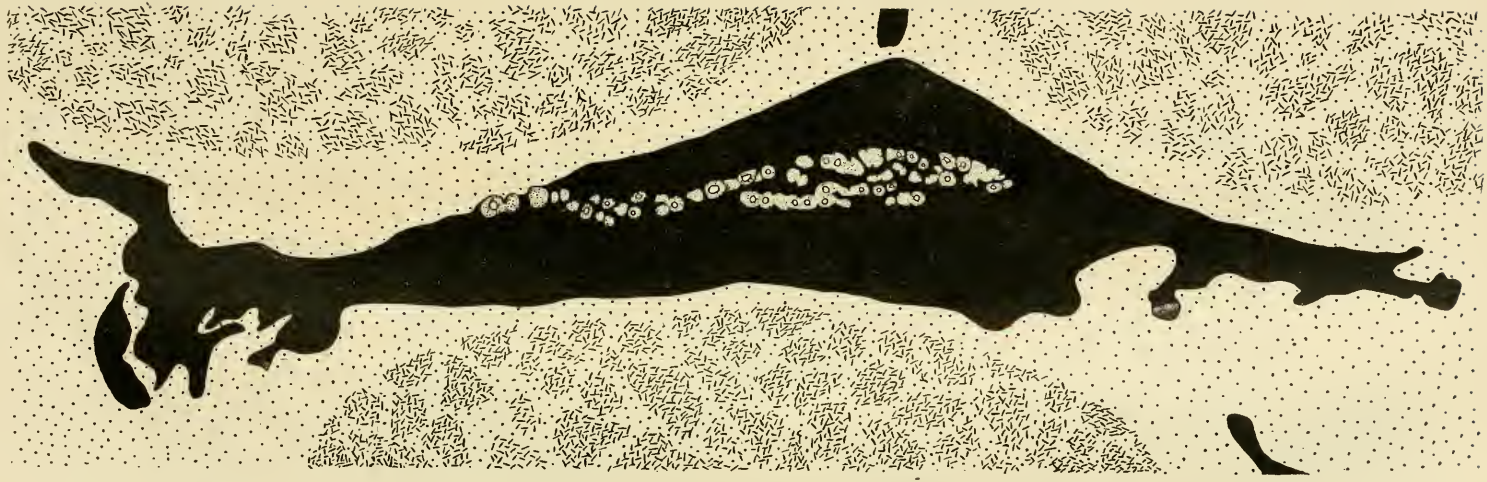

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Figs. 8 to 14 A group of semidiagrammatic drawings (camera lucida outlines) showing the vascular relations of the thyreoid during the successive stages of its morphogenesis. The vascular elements are shown in solid black and the epithelial portions in a fine close stipple. Mesenchyme is shown in loose stipple; muscle in lines; cartilage in coarse stipple. × 45.

Fig. 8 Cross-section through the pharyngeal region of an Acanthias embryo 5.2 mm. long (No. 7). The thyreoid bud is shown attached to the ventral wall of the pharynx. The first aortic arch of either side lie in contact with the lateral surfaces of the bud. The anterior head cavities lie lateral to the aortic arches. The pharynx, the second gill clefts, the dorsal aortae, and the lower half of the notochord are also indicated.

Fig. 9 Cross-section through the mandibular process of an Acanthias embryo 14 mm. long (No. 24). The thyreoid gland is shown as a pedunculated bud attached to the pharyngeal floor. The aortic arches are separated from the thyreoid bud by a considerable mass of mesenchyma. Two anterior head cavities are located ventrolaterally to the thyreoid.

Fig. 10 Cross- section through the ventral wall of the pharynx of an Acanthias embryo 28 mm. long (No. 38). Thyreoid gland is shown near the center of the figure as a flattened structure having two closed cavities within. A considerable amount of mesenchyma is present in which blood-vessels, muscles, and cartilage are being developed. The vessels are a part of the thyreoid plexus of venules. The most ventral circular muscle is the constrictor pharyngis. The longitudinal bundle just ventral to the thyreoid is the coraco-mandibularis. The dense mesenchyma dorsal to the thyreoid is the anlage of the basihyal cartilage. The most dorsal and ventral structures are entoderm and ectoderm, respectively.

Fig. 11 Cross-section of the ventral wall of the pharynx of an Acanthias embryo 33 mm. long (No. 40). Thyreoid gland is shown near the center of the figure as a flattened structure, having four cavities, all of which are invaded by blood-vessels. Blood-vessels have increased in size and number around the gland and have associated themselves more closely with its surfaces. The surfaces of the epithelial plates of the thyreoid are quite smooth. The ectoderm, constrictor pharyngis, and coracomandibularis muscles, the basihyal cartilage, and the entoderm have positions similar to those of the same structures in figure 10.

Fig. 12 Cross-section of a part of the ventral wall of the pharynx of an Acanthias embryo 48 mm. long (No. 45). Thyreoid gland is shown near the center of the figure as a structure made up of anastomosing epithelial plates which are quite regular in thickness and which have smooth surfaces. Blood-vessels have increased in size and number around the gland and have intimately associated themselves with its surfaces. The other structures shown are located similarly to the same structures in figure 11, with one exception. The left coracohyoid muscle is located between the thyreoid gland below and the basihyal cartilage above.

Fig. 13 Cross-section of a part of the ventral wall of the pharynx of an Acanthias embryo 95 mm. long (No. 49.). Thyreoid gland is shown near the center of the figure as a structure which is made up of epithelial plates which have rough surfaces. Follicles are being formed in the plates and a few follicular lumina may be seen. The blood-vessels have fused around the gland to form the thyreoid sinus within which the gland is suspended. Note the two valve leaflets at the left end of the sinus. The other structures shown are located similarly to the same structures in figure 11.

Fig. 14 Cross-section of a part of the ventral wall of the pharynx of a newborn specimen of Squalus acanthias 170 mm. long (No. 52). The thyreoid gland is shown near the center of the figure as a structure which is made up of follicles and a few plates in which follicles are being formed. The magnitude of the thyreoid sinus and the fact that the thyreoid is suspended within it from only two points are striking features. Note the valve leaflet at the left end of the sinus. The other structures shown are located similarly to the same structures in figure 11.

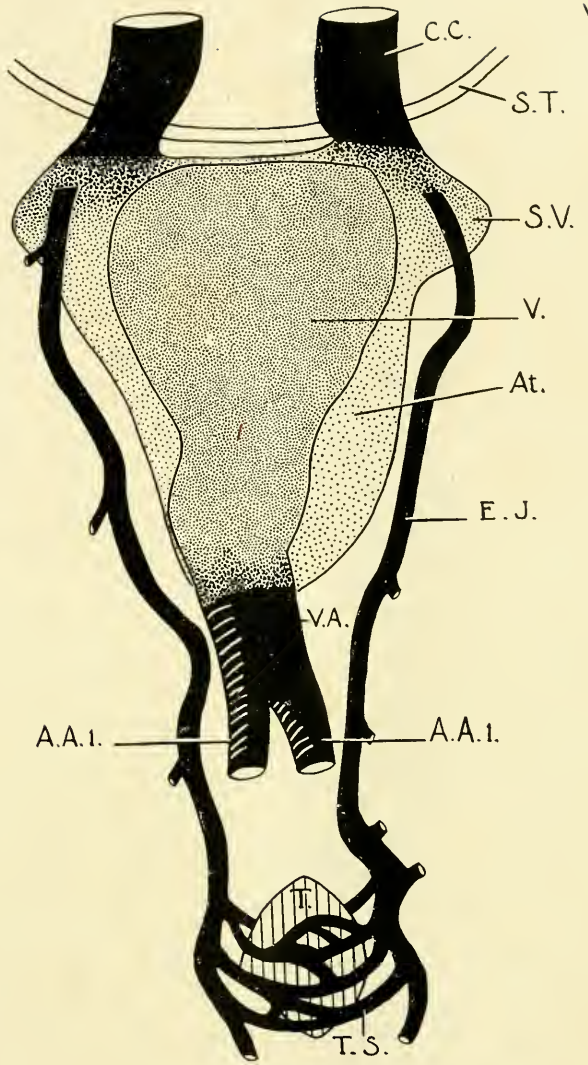

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Fig. 15 Semidiagrammatic graphic reconstruction of the thyreoid gland and related vascular elements of an Acanthias embryo 28 mm. long (No. 38), seen from the ventral aspect. *A.A.1*, first aortic arch; *At.*, atrium, *C.C.*, common cardinal vein; *E.J.*, external jugular; *S.T.*, septum transversum; *S.V.*, sinus venosus; *T.*, thyreoid gland; *T.S.*, thyreoid sinusoids; *V.*, ventricle; *V.A.*, ventral aorta. × 8.

6. DISCUSSION AND CONCLUSIONS

The form of the thyreoid anlage in Squalus acanthias is of interest. As pointed out in the section on organogenesis, the anlage has the form of a localized, bud-like thickening of the pharyngeal epithelium (figs. 1, 2, 17). There is no thyreoid pouch or 'thyreoglossal duct' (His, '91) developed in any member of the present series, as has been described by W. Müller ('71) in an Acanthias embryo and by other observers in other forms. This may be a developmental peculiarity or, what seems more probable, it may be that the relative growth changes in the cervical region of the Acanthias embryo are less marked than they are in the same region of various other vertebrates, so that instead of being forced to change its relative position and to draw out a long connecting stalk, the gland remains in close relation to the pharyngeal floor until after its separation. The small mound-like projection from the dorsal aspect of the body of the gland, found in specimens immediately following the separation stage, probably marks the point of glandular detachment and may represent the thyreoglossal duct of other forms.

The fact that the relative position of the gland is changed very little is clearly shown in figure 16 of the present paper and also in a figure (fig. 3) published by Camp ('17). These figures indicate that the principal topographical change is brought about through the outgrowth of the long caudal portion of the gland, and that, although there is a small amount of growth forward from the anterior end of the body, there is almost no shifting of the point of detachment from the level at which the gland was originally set free. In other words, this point of detachment seems to remain through successive stages at a point about midway between the first and second gill clefts.

Goodey ('10) studied the relations of the adult thyreoid gland in three species of Selachians (Chlamdoselachus angineus, one specimen; Scyllium catulus, three specimens; Scyllium canicula, about thirty specimens) which are closely allied biologically to Squalus acanthias. In some of these he found varying degrees of connection between the thyreoid gland and the pharynx. In

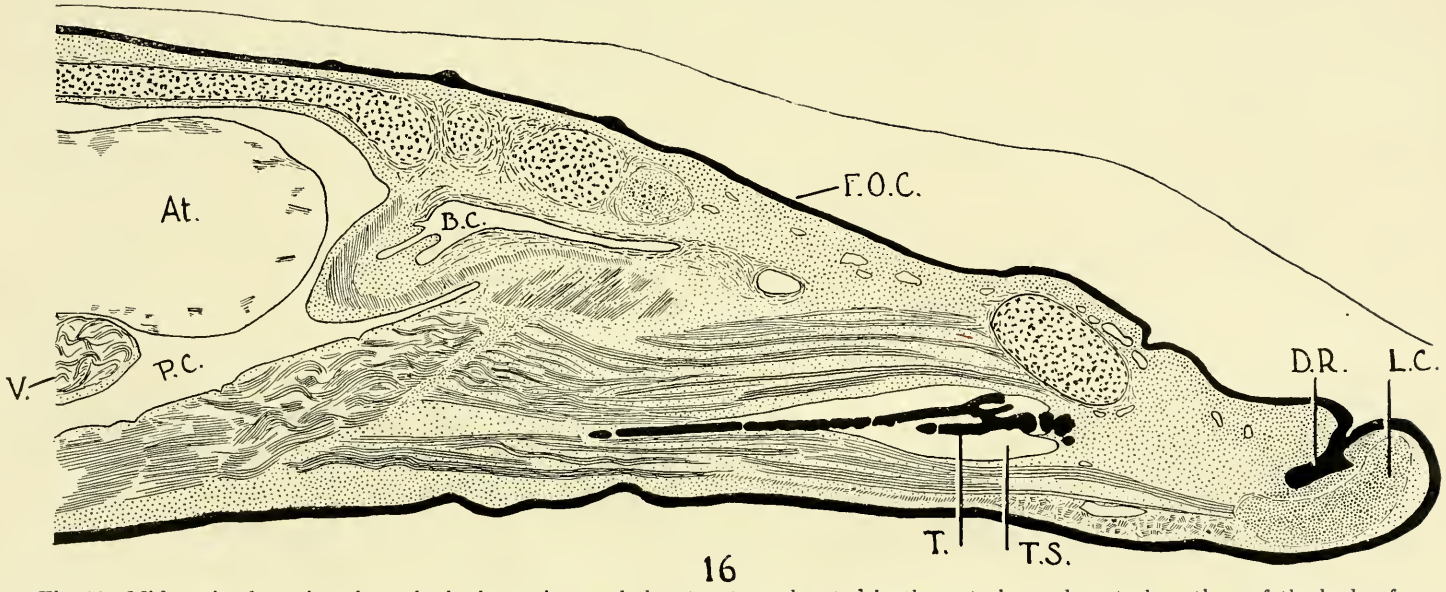

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Fig. 16 Midsaggittal section through the lower jaw and the structures located in the anterior and ventral portions of the body of an Acanthias embryo 60 mm. long (No. 47), showing the topography of the thyreoid gland. *At.*, atrium; *B.C.*, bulbus cordis; *B-h.C.*, basihyal cartilage; *D.R.*, dental ridge; *F.O.C.*, floor of oral cavity; *P.C.*, pericardial cavity; *T.*, thyreoid gland; *T.S.*, thyreoid sinus; *V.*, ventricle. × 25.

one case he found a tube-like structure extending from the pharynx through a foramen in the basihyal cartilage to the anterior end of the thyreoid, and in three other cases he found typical thyreoid tissue within the foramen of the basihyal cartilage. He concludes that this foramen "must have been occupied by the original entodermic evagination." These results of Goodey receive no support from the present investigation, for the thyreoid gland in Squalus acanthias has completely severed its connection with the pharynx some time before the basihyal cartilage is laid down. However, certain possibilities suggest themselves in attempting to harmonize these apparently conflicting observations. It may be that the thyreoid of the forms described by Goodey actually undergoes a somewhat different developmental history from that described in the present paper, or it may be that the cases in which he found adventitious thyreoid tissue are exceptional and represent cases of delayed separation of the gland.

The transformation of the gland from its columnar form (figs. 4, 19) to a plate-like structure, flattened dorsoventrally (figs. 5, 10, 11, 20, 21, 22, 23), is apparently due to pressure exerted by the development of surrounding elements, the chief of which are the basihyal cartilage above and the ventral musculature below.

What may be the significance of the vacuole-like spaces which appear within the early gland (fig. 3) cannot be said. Also an explanation of the meaning of the difference in form of the nuclei of the central and peripheral portions of the gland in these early stages (figs. 3, 4) is wanting. Balfour ('85), in describing the structure of the thyreoid of a Scyllium embryo which belongs in his stage L, points out a similar arrangement of the cells, but offers no suggestion of its meaning.

The large amount of granular pigment found in the parenchyma of the gland is a striking feature (fig. 4). This pigment increases in amount up to the time of separation of the gland from the pharynx, and thereafter rapidly disappears. It is massed in largest quantity at the point at which separation is to take place. Similar pigment is to be seen in the ectoderm ventral to the thyreoid region, and was also described by Balfour ('85) in

the thyreoid of Scyllium embryos, and figured by Baumgartner ('15) in the hypophysis of Squalus acanthias. The pigment does not seem to be associated with nuclear or cellular degeneration, although in some cases the pigment blocks are superimposed on, and nearly obscure certain nuclei. The fact that this material is found in the ectoderm in the region near which the oral plate has opened, and in the thyreoid, chiefly in the neck region of the gland, suggests that it may be associated with any division or separation of epithelia.

After the gland has established its independence from the pharynx, its structure is again altered, as described above, by the development of completely closed cavities within the thicker parts of the gland mass. These cavities, although not previously described in the Selachian thyreoid, apparently correspond to the lumen which W. Müller ('71) mentions as present in the thyreoid of one of his Acanthias embryos, and to the cavities which Scammon ('11) observed in three specimens in the Normal-plate series (No. 29, 31, 32).

Since the gland has been regularly found to be solid up to the time of its separation from the pharynx, and since no thyreoid pouch or thyreoglossal duct has been observed, it is evident that these cavities develop quite independently of any earlier pouch, duct, or cavity. Further evidence in favor of this conclusion may be drawn from the fact that a number of these closed cavities or spaces of various sizes have been found in different stages of development not only in many of the glands, but also in different parts of the gland in the same specimen.

The cavities, which I first observed in the human thyreoid (Norris, '18) have been subsequently found in the thyreoid of Acanthias embryos (Norris, '17). In the human embryos, the cavities appear at a stage in the glandular development which exactly corresponds to the stage of their genesis in the fish thyreoid. Moreover, the rôle they play in the two forms is apparently identical. Born ('83), in describing the thyreoid glands of pig embryos 7 mm. long, mentions the presence of lumina in the lateral end of the gland mass. He does not include any description of these lumina, but they probably correspond to

the cavities described in this paper. Since finding these intraglandular spaces in the thyreoids of human embryos and in those of Squalus acanthias embryos, I have made a brief study of a number of intermediate forms (pig, sheep, dog, pigeon) and have found similar cavities present in the thyreoid of each form investigated. It is hoped that these observations may be extended and presented in a subsequent publication. The existence of this same feature of morphogenesis in the thyreoid glands of two animal groups as far removed from one another as fish and man, as well as in the glands of a number of intermediate forms, will probably justify the conclusion that this is a fundamental feature of thyreoid development.

Any attempt to interpret these early intraglandular spaces either on the basis of their immediate or general biologic significance, although interesting, can be at best only speculative. It might be thought that the immediate purpose and significance of these cavities is to be found in the formation of the two-celled plates from which the follicles are later to be derived. On the other hand, if the vertebrate thyreoid is the phylogenetic representative of a true externally secreting gland (Patten, '17), it might be suggested that these cavities appear in response to a tendency to reproduce the ancient lumen or duct of the ancestral gland. This theory is attractive, inasmuch as it permits of harmonizing the known facts, both anatomical and physiological, regarding the endocrine function of the thyreoid. From this point of view the hypothesis and conclusion of Bensley ('16), 'that the thyroid cell represents a true reversal of polarity,' would be quite unnecessary. On the other hand, this phylogenetic theory would offer a plausible explanation of the present structure of the gland and its endocrine function without making it essential to hypothesize a reversal of cellular polarity.

It might be objected that the fact that these intraglandular spaces have no relation to the lumen of the thyreoglossal duct would appear to be a serious obstacle to the theory just advanced. But such a contention is not as formidable as it might seem at first sight, when it is considered that in the form at present under discussion no thyreoglossal duct is ever formed, and, moreover,

even in those forms in which such a structure is sometimes developed, there are no specific cases described in which the lumen of the thyreoglossal duct is continuous with a cavity in the gland-mass proper. In other words, since the lumen of the duct does not extend into the body of the gland, any cavities found there must be morphologically independent of it, although they may, as the theory would suggest, be phylogenetically related.

The development of intra-epithelial clefts and spaces is not unusual. The lumina of a number of the true glands are formed within solid epithelial sprouts and buds which are the anlagen of the future ducts and tubules. Again, the vacuoles in the epithelium of the oesophagus and duodenum which were described in detail by Johnson ('10) are striking examples of this same feature of morphogenesis. In all these instances, however, the spaces ultimately form a connected system of cavities or open into a common lumen. The intra-epithelial cavities of the early thyreoid gland, on the other hand, have a very different fate. They open more or less independently of one another to the outside of the gland mass and are then invaded by the surrounding vascular mesenchyme. Such a process apparently has not been observed in the morphogenesis of any other organ and seems to be quite unique and peculiar to the thyreoid gland.

Before attempting to discuss the follicular period, the fact must be emphasized that the cavities or lumina of the thyreoid follicles are entirely independent of the earlier transient intra-glandular cavities described in the present paper.

Remak's ('55) theory of the derivation of the thyreoid follicles directly from a primitive saccular thyreoid anlage has not been confirmed. In the prefollicular stages, the thyreoid is by recent investigators quite generally described as assuming the form of irregular, anastomosing 'cords' or masses of epithelium. This undoubtedly appears to be the case when sections of the gland are observed (figs. 11, 12, 13). But the reconstruction methods used in the present investigation reveal a surprisingly different condition. It is found that, as a matter of fact, in the great majority of cases the cords are illusions and in reality are merely sections of fenestrated epithelial plates longitudinally arranged.

As to the further steps in the process of morphogenesis of the follicles from these anastomosing 'cords,' widely divergent views have been held. Inasmuch as the process of follicle genesis has been found to be practically identical in Squalus acanthias with the process described by the author in the human thyreoid (Norris, '16), the reader is referred to this earlier work for the discussion of the changes noted and for a consideration of the various views presented by the literature as regards this matter.

The relative magnitude of the vascular bed which is associated with the gland, the close relationship which is established between the blood stream and the parenchymatous elements, and the comparatively small amount of arterial blood furnished to the gland are the most noteworthy conditions in connection with the blood supply of the thyreoid.

As shown in figure 14, the gland is literally suspended in a lake of blood, the blood being separated from the epithelial structure only by a single layer of epithelial cells.

7. SUMMARY

1. The thyreoid gland in Squalus acanthias makes its appearance in embryos of approximately 4 mm. in length, as a solid epithelial bud from the floor of the pharynx.

2. The bud grows caudally and becomes keel-shaped. At this stage it is joined to the pharynx by a very short and narrow stalk.

3. By the time the embryo has attained a length of 19 mm. the gland has severed its connection with the pharynx and has the form of a column with rounded ends.

4. In no case was a 'thyreoglossal duct' observed.

5. The gland soon loses its columnar form, and as it gradually flattens out dorsoventrally, becomes lozenge-shaped in outline.

6. In embryos of about 30 mm. the gland for the first time assumes in miniature the form of the adult gland. It may be divided for descriptive purposes into two portions: an anterior part (corpus), which is rhomboidal in outline and relatively thick in cross-section, and a posterior part (cauda), which is relatively thin in cross-section and has the form of a long straight bar

joined with the posterior angle of the corpus in front and terminated caudally in a pointed extremity.

7. Except for the indefinite vacuole-like spaces which are found in the thyreoid of embryos ranging from 5 to 20 mm. in length, the gland is quite solid throughout the period of its early morphogenesis.

8. At about the stage (20 mm.) when the vacuole-like spaces cease to be found, there are formed within the thicker portions of the gland a number of completely closed cavities, which in contrast to the earlier vacuole-like spaces are outlined by very definite margins. These intraglandular cavities are very tiny when they first appear, but as they increase in size they may open into one another, thus transforming the gland from a solid into a hollow organ.

9. At a later stage (28 mm.) these cavities for the first time open to the outside and are invaded by blood-vessels.

10. The process of cavity formation is accompanied or perhaps preceded by four other processes, as follows: 1) differentiation of cell boundaries; 2) changing form of the nuclei (cells); 3) changed position of the nuclei (cells); 4) cell proliferation.

11. The intraglandular cavities are regularly surrounded by epithelial plates which are two cells in thickness, so that when the cavities are opened to the outside the gland is transformed into a number of smooth epithelial plates which anastomose with each other freely and which are regularly two cells thick.

12. The primary thyreoid follicles arise directly as isolated and independent structures from the epithelial plates of the prefollicular period, by the rearrangement of cells, cell proliferation, increase in the size of the cells, and lumen formation.

13. The primary follicles appear in embryos of about 60 mm. in length and continue to be formed until the embryo attains a length of approximately 250 mm.

14. There are no secondary follicles found before 250 mm.

15. Branches of the external jugular vein invade the region of the thyreoid and begin to form a vascular plexus around the gland in embryos about 19 mm. in length.

16. The elements of this venous plexus associate themselves intimately with the parenchymatous elements. The thyreoid sinus is formed by the increase in their size and by their fusion with one another.

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PLATE 1

EXPLANATION OF FIGURES

17 Left lateral view of a wax reconstruction of the pharynx of an Acanthias embryo 5 mm. long (No. 6). *E.*1 and *E.*2, ectodermal plates of the first and second gill clefts, respectively. These plates are cut away arbitrarily around their edges. *G.P.* 3, third gill pouch which has not yet fused with the ectoderm; *Nc.*, notochord; *O.Pl.*, oral plate; *T.*, thyreoid. × 75.

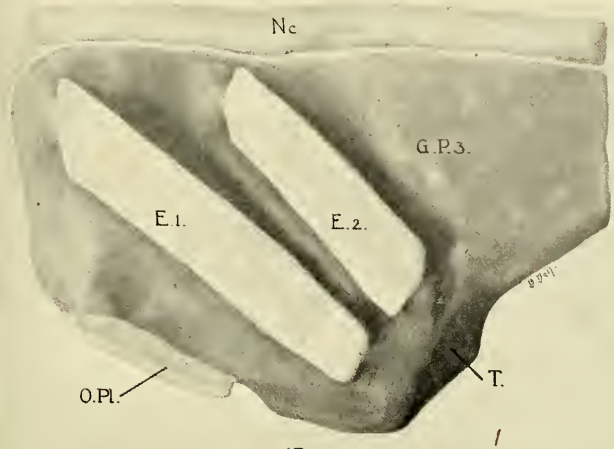
18 Right lateral view of a wax reconstruction of the pharyngeal floor of an Acanthias embryo 14 mm. long (No. 24). *G.P.*1 and *G.P*2, first and second gill pouches, respectively; *O.Pl.*, oral plate; *T.*, thyreoid. × 100.

19 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 21 mm. long (No. 34). × 100.

20 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 24 mm. long (No. 36). × 100.

21 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 28 mm. long (No. 38). × 100.

In figures 20, 21, and 22 the dark areas represent the position of intraglandular cavities whose outlines have been projected on the ventral surface of the gland.



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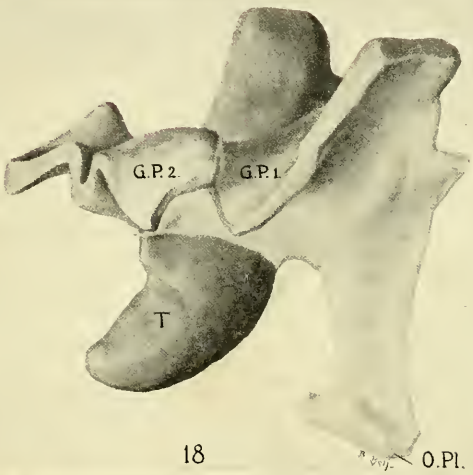

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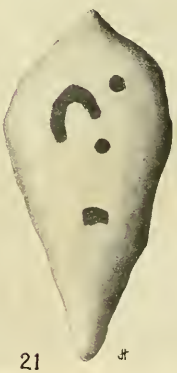

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PLATE 2

EXPLANATION OF FIGURES

22 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 36 mm. long (No. 42). The anterior rhomboidal corpus and the posterior elongated cauda are easily recognizable. The dark area on the corpus represents the position of the intraglandular cavity whose outline has been projected on the ventral surface of the gland. This cavity has opened to the outside on the dorsal aspect of the gland. Its opening is not indicated in the figure. × 100.

23 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 60 mm. long (No. 47). The roughness on the surface is due to the beginning formation of follicles in the epithelial plates. The clear areas represent perforations or fenestrae in the plates produced by the gradual separation of the forming follicles. The points at which the intraglandular cavities have opened to the outside are also seen. × 65.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

A STUDY OF THE RELATION OF THE BEHAVIOR OF THE CHROMATIN TO DEVELOPMENT AND HEREDITY IN TELEOST HYBRIDS[1]

EDITH PINNEY

EIGHTY-EIGHT FIGURES

INTRODUCTION

The facility with which crosses between distantly related species of teleosts can be made and the varied results obtained from such crosses offer a problem which has received much attention from zoologists in recent years. Although the researches of Moenkhaus, Newman, the Hertwigs, and others have contributed to the solution of certain phases of the problem which concern the unlike results of reciprocal crosses and the differences in development shown by the individuals of any one cross, the explanations of these workers are more in the nature of suggested hypotheses than proved theories. It was in view of the need for further investigation of the cytology of these hybrids that this study, which deals with the behavior of the chromosomes of several heterogeneous crosses, was undertaken.

The behavior of the chromatin in teleost hybrids was first described by Moenkhaus in 1904 in reciprocal crosses between Fundulus heteroclitus and Menidia notata. He demonstrated that the chromosomes of both of these species retained their morphological characteristics when brought together by cross fertilization, and his results afford splendid support to Boveri's hypothesis of the individuality of the chromosomes. None of the hybrids survived the early germ-ring stages and, although the chromatin was followed through several of the early and the

[1] A dissertation presented to the faculty of the Graduate School of Bryn Mawr College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. For assistance in publishing the illustrations accompanying this paper I am indebted to the Naples Table Association for Promoting Laboratory Research by Women.

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late cleavage stages, no attempt was made to explain the developmental abnormalities in the hybrids on the basis of the behavior of the chromatin.

The first attempt at such an analytical comparison of developmental and cytological processes is that of Günther and Paula Hertwig, whose results were published in February, 1914. These investigators made, among many other teleost crosses, six that were heterogeneous. The results of all of their experiments, although extremely diverse, are thought by them to be due primarily to a disharmony of idioplasms as set forth by O. Hertwig: "Die idioplasmatische Disharmonie beruht auf der verschiedenen materiellen Beschaffenheit der mütterlichen und väterlichen Kernsubstanzen und ist von dem Grade der Verwandtschaft zwischen den beiden zum Bastard verbundenen Stammformen abhängig." They recognize that this alone cannot explain the unequal success of reciprocal crosses. They therefore assume a further disharmony between the cytoplasm of the egg and idioplasm of the spermatozoön, a relation which is not necessarily the same in reciprocal hybrids. Although the main conclusion of their work is that the disturbances in development are due to the combined effects of disharmonies, first, between the nuclear constituents of egg and spermatozoon, and, second, between the cytoplasm of the egg and the spermatozoon, nothing abnormal was found in the behavior of the nuclear substance until very late embryonic stages when the nuclei are said to be abnormally large. They did, however, obtain some evidence of a specific effect of the cytoplasm.

The observations of Miss Morris ('14), on the cross Fundulus heteroclitus ♀ × Ctenolabrus adspersus ♂ are especially interesting in this connection, since this is a cross which gives purely maternal larvae. Miss Morris found two types of chromosomes in the early cleavage stages of the hybrids which she identifies as the two paternal types. The slight lagging of the foreign chromatin does not, she thinks, result in any elimination of chromosomes and all of the maternal and paternal chromatin participates in mitosis. She rejects Loeb's theory ('12 a), "That the function of the sperm in such hybrids is merely that of a parthenogenetic agent," and concludes that the Fundulus

egg fertilized with the sperm of Ctenolabrus gives purely maternal larvae without elimination of the foreign chromatin.

Newman, too, believes that larvae which are phenotypically pure maternal, still bear in their germ plasms the "full complement of paternal inheritance factors." Referring to such cases as the above, he says, "We are therefore not dealing with a case of parthenogenetic development, but with one involving a more or less complete dominance of the egg over the sperm." This and other of Newman's views may be more profitably discussed later.

Miss Morris' description of the Fundulus chromosomes agrees with that of Moenkhaus, but, as I will show, she was in error as regards the smaller chromosomes of Ctenolabrus. Ctenolabrus chromosomes are rods, some of which have a length which is several times that of their width. Many of the shorter elements are hook-shaped, recalling one type of Echinoderm chromosome, and a very few are so short as to appear almost round, but their width does not exceed that of the longer rods. It is possible that a pair of small V-shaped chromosomes are present. The width of the rod-shaped elements is exceeded only when the rod is bent on itself to form hooks or V's.

My observations on the straight fertilized Ctenolabrus egg, made in the summer of 1914 in connection with another problem, suggested to me the possibility that the Ctenolabrus chromosomes in Fundulus eggs observed by Miss Morris were abnormal as evidenced by their change in shape, and that this perhaps accounted for their ineffectiveness in this cross. In the hope of adding to our knowledge concerning the rôle of the chromosomes in this and other Teleost crosses, I began the investigations, the results of which are given in this paper.

I am indebted to Professor D. H. Tennent for directing my attention to this field of work and it gives me much pleasure to express here my deep appreciation of his many kindnesses throughout the course of my investigations. For the privilege of working at the Marine Biological Laboratory my thanks are due both to Professor Frank R. Lillie, the director of the laboratory, and to Bryn Mawr College. I am especially grateful to Mr. G. M. Gray for his valuable assistance in supplying me with material.

MATERIAL AND METHODS

The material for this study comprises many series of eggs obtained from heterogenic crosses and fixed at successive stages in development. Each cross was repeated as often as possible in order to insure appropriate stages. Series of straight fertilized eggs of all of the species used were also fixed for comparison with the hybrids. The familiar methods of making the straight and cross fertilizations were used and the usual precautions to prevent undesired chance fertilizations were strictly followed. In connection with every experiment unfertilized controls were kept. In no case did these controls show dividing eggs. It is to be regretted that in all cases the development was not followed to the end, but, owing to the abundance of available data in regard to development in fish hybrids, this was not thought necessary.

Bouin's, Perenyi's, Gilson's, and Kleinenberg's fixatives were used. The Bouin fixative gave uniformly good results. The fixation with Perenyi proved to be very capricious. Eggs, fixed in Perenyi's fluid in July, 1914, and kept in alcohol for over a year and a half, afforded cytological material which from the standpoint of fixation left nothing to be desired. In view of the splendid results of its first trial, I used it freely and with great confidence in preserving material in the summer of 1916. None of the later material killed in Perenyi has been well fixed. By its action the entire system of astral fibers which extends throughout the cell during the later mitotic phases is often entirely obliterated. More frequently the spindle remains, but its fibers are twisted and distorted and the chromosomes are hopelessly massed together. The best results obtained were when the eggs were fixed during anaphase stages. Such material affords good evidence on some points. The original plan was to fix parallel series, using at least two fixatives, but it was soon found that the amount of available material did not permit this, and the plan was abandoned. The best that could be done was to use one fixative for an entire series and supplement this with occasional stages fixed in some other fluid. In cases where Perenyi's fluid was used for the main series the number of well-fixed stages is small.

Most of the observations recorded here were made on material fixed with the fluid of Bouin. This gave splendid results when used for the smaller pelagic eggs of Ctenolabrus and Stenotomus. In the larger eggs of Fundulus and Menidia the structural details of the cytoplasm and the archoplasmic fibers are coarser and the spindles are smaller. This, I think, accounts largely for the fact that the chromosomal images during the anaphase are somewhat less clear than in the smaller eggs.

The large eggs of Fundulus and Menidia are easily dissected from the chorion and can either be imbedded and sectioned separately in the manner described by Moenkhaus, or the blastoderms can be removed from the yolk and numbers of them imbedded together. The latter method, while demanding larger quantities of material, is convenient in that one obtains sections in many different planes in less time than by the other method. This is an especially good way to handle the smaller pelagic eggs. The task of removing the egg from the membrane is somewhat more tedious in this case, but if executed under a binocular is not difficult. It is well to remove as much as possible of the yolk from the blastoderms, for, although it imbeds and sections well, during the process of staining bits of it are easily dislodged and scattered over the surface of the sections where they may prove bothersome. In the early stages, just after fertilization, the germ disc is not formed, and such eggs must be imbedded whole after removing the chorion.

Sections were cut five or six microns in thickness and stained with Heidenhain's iron-haematoxylin. Bordeaux red was used in some earlier preparations as a counterstain, but, as it seemed to have no particular advantage, its use was discontinued.

Such experimental data as is thought necessary will accompany the cytological observations on the corresponding material and each cross will be described separately. The conclusions drawn from the separate crosses will be reserved for the general discussion of the results.

Fundulus heteroclitus ♀ × *Fundulus heteroclitus* ♂

My observations on the Fundulus egg concern only the number and form of the chromosomes and agree in the main with those of previous observers.

The typical long thin rods are shown in figures 1 and 2. The straight ranks of chromosomes in anaphase stages are more striking in the object itself than in the drawings. The drawings show only those rods which could be brought wholly into view by careful focusing.

All of the chromosomes in such lateral views cannot be seen distinctly. The reason for this will be obvious from figure 3 which is a polar view of an anaphase plate. Forty-five chromosomes were counted, all of which appeared in one section.

This figure was found after many mitotic figures of the hybrid eggs of the cross Ctenolabrus adspersus ♀ × Fundulus heteroclitus ♂ had been examined. Having in mind the statement of Moenkhaus that "In a given anaphase all of the chromosomes are of practically the same length," I tried to estimate the number of chromosomes characteristic of Fundulus by counting the longest rods in these hybrids where the long rods are more conspicuous than in a straight fertilized Fundulus egg. My counts varied from fourteen to sixteen.

Therefore, the large number met with in polar views was surprising, but further study of Fundulus material has shown that the earlier misconception was probably due to the hitherto unsuspected presence on the Fundulus spindle of shorter chromosomes. Figure 4, which gives two sections of an early anaphase, shows this to be the case. Not all of the chromosomes have divided. On some parts of the spindle they are crowded together and separate chromosomes cannot be distinguished. As a result of this, some elements are unusually clear and the presence of shorter rods is demonstrated beyond question. Only the clearest rods were drawn and all of the shorter ones lay in the section and not at the surface. This excludes the possibility of their being sections of longer rods. Figure 5 is of a slightly earlier anaphase. It will be noticed that the shorter

rods complete their division first. That in figures of later anaphases the shorter chromosomes had long escaped observation is not surprising in view of the compact arrangement of the daughter plates already emphasized.

Ctenolabrus adspersus ♀ × *Ctenolabrus adspersus* ♂

The Ctenolabrus egg is the smallest of the pelagic eggs used. Its transparency, which permits the progress of segmentation to be followed easily, and the rapidity with which it develops make it a favorite object for embryological study.

The fish are small and quite easily handled and mature females yield a large number of eggs, which is an added advantage. The eggs are very susceptible to adverse conditions, and numbers in every lot were evidently injured during the process of stripping and addition of the sperm. The eggs were stripped into finger bowls containing a slight quantity of sea-water, the sperm added and stirred thoroughly among the eggs. After one or two minutes more sea-water was added. The eggs surviving this manipulation were skimmed off into bowls of fresh sea-water. The first cleavage occurs about forty to fifty minutes after fertilization, but no careful observations were made on the cleavage rate.

Reference has been made in the introductory section of this paper to the nature of the chromosomes in the Ctenolabrus egg fertilized by spermatozoa of its own species. The elongated rod-like form of these bodies is evident. They have little resemblance to the metaphase chromosome in figure 9 of Miss Morris's paper. Figure 7 shows, in three sections, an early anaphase of the fourth cleavage. A slight twisting of the spindle fibers has resulted in obscuring certain elements while leaving others well exposed to view. Straight rods, hooks, and two thicker bodies, which I interpret as V-shaped chromosomes, stand out clearly. Comparison with figure 4 shows that the longest rods of Ctenolabrus approximate in length the shortest rods of Fundulus. The spindle is also broader and the individual chromosomes more widely separated, hence the band-

like effect obtained in a side view of an anaphase plate is not so marked as in Fundulus.

Figure 8 is a drawing of a much later anaphase of the second cleavage. The same forms appear. A few chromosomes were displaced by the knife in sectioning. Figures 9 and 10 of the fourth and second cleavage stages respectively show the same features.

No very satisfactory polar views of anaphase spindles were found and no very reliable counts of the chromosomes could be made. Figures 11, 12, and 13 represent such views as were obtained. In these the chromosomes of each polar group are distributed through two sections. The counts are thirty-eight, forty-one, and forty-four, respectively. These are plates from fourth-cleavage spindles. Figures 14 and 15 are similar groups from second-cleavage spindles. One shows forty-four and the other forty-eight bodies. It is apparent that an exact estimate of the number of chromosomes present in a fertilized Ctenolabrus egg cannot be made from this evidence, but the results are given here because it is thought that they are of interest in that they give an approximate idea of the number of chromosomes involved in any cross made with Ctenolabrus.

The chromosomes from one section of a telophase group are shown in figure 16. The formation of vesicles has already begun. This process is essentially similar to that described by Richards ('17) for Fundulus. The chromatin rod lengthens by the separation of its constituent chromomeres. The end of the rod nearest the pole swells first, indicating that the liquid which they absorb comes from the region of the centrosphere. Figure 17 shows the young nucleus consisting of individual vesicles. I have not tried to discover whether or not these vesicles remain separate during the phases of the resting nucleus.

Fundulus heteroclitus ♀ × *Ctenolabrus adspersus* ♂.

After comparing the chromosomes of Ctenolabrus with those of this hybrid as Miss Morris has figured them, one is forced to the conclusion that the behavior of these 'bearers of heredity'

in the strange environment of the Fundulus egg cannot be characterized justly as 'normal.' All of her drawings of the hybrids show lagging of the chromosomes in the anaphases, which in itself is a marked irregularity. It is quite conceivable that a slight lagging may not preclude normal division, but that such a condition as her figures 12, 17, and 19 show, always leads to an equal distribution of the chromatin to the two poles of the spindle is doubtful. The altered form of the Ctenolabrus chromosomes is difficult to interpret. At first it seemed that it might be regarded as a sign of degeneration, but, after examining some of the same hybrid eggs in which shorter Ctenolabrus rods are found that are quite normal in appearance, it is evident that, if degeneration does occur, it does not affect all of the chromosomes. It was not my intention to make an extended study of this cross, therefore I preserved very little of the material, but such as I have presents a few facts which seem worthy of mention, since they give evidence of abnormal mitoses occurring in these hybrids. From the following description it will be seen that these abnormalities take the form of an exaggerated lagging of the chromosomes during division, which probably results in the elimination of whole chromosomes from the nucleus.

Figures 18 and 19 are sections of the two anaphase spindles of one egg. The sections are oblique to the axis of the spindles, but the corresponding poles are lettered alike in each drawing so that one can obtain a fair idea of the nature of the elements gathered at either pole and scattered between. Definite, blackly staining bodies occur at the equatorial plate of each spindle, although the mass of this material is greater in the spindle of figure 18 than in the other. These bodies have a very similar appearance to that of the yolk bodies scattered through the cytoplasm. They have the same homogeneous consistency, the smooth contour, and in some instances the spherical form. Slender protuberances from these masses resemble the ends of chromosomes. Whether the other end is fused with the larger body or is merely hidden by it, is impossible to determine. Normal Fundulus chromosomes and some apparently normal Ctenolabrus chromosomes appear at the poles. Some abnormally thickened bodies are present which suggest undivided chromosomes.

The spindles of mitotic figures in normal eggs of these two species differ. In Fundulus, as the chromosomes are drawn to the poles, they are crowded closely together and the polar ends of the spindle are constricted (figs. 1 and 2). In Ctenolabrus the separating groups of chromosomes occupy a broader field, approximating in area that of the equatorial plate, and therefore the anaphase spindles are more uniform in diameter throughout their extent than is the case in Fundulus (figs. 8 to 10). An idea of the relative areas occupied by the anaphase groups of chromosomes in both species may be obtained by a comparison of figure 3 with figures 11 to 15. This difference in the physical character of the spindles may account for the interference with the normal division of the Ctenolabrus chromosomes in the Fundulus egg. As will be seen later, in the reciprocal cross with the Ctenolabrus egg, a similar disturbance during division is exceedingly rare. Moenkhaus found that when the egg of Fundulus was fertilized by the spermatozoa of Menidia, the early cleavage divisions were normal; there was no lagging. He reports, however, the presence of only thirty-six chromosomes in Menidia, and it is possible that the mechanical difficulties offered by the small Fundulus spindle are not so great when this species is used in crossing as they are when the sperm of Ctenolabrus is used.

The few late telophases of the first cleavage that were found exhibited no atypical features. Nothing that could be identified as lagging chromosomes was observed. There are two features of the Fundulus egg that might prevent the recognition of extra-nuclear chromatin if it did occur. These are: first, numerous large yolk granules scattered throughout the cytoplasm which vary greatly in their affinity for the chromatin stain, and, second, the very coarsely reticular character of the cytoplasm itself, the meshes of which (and this is particularly true in the region of the first cleavage plane) are very similar to the chromosomal vesicles.

Figures 20 to 25 show what appears to be an active elimination of chromosomes from the nucleus. Figure 20 presents a cell in the metaphase of the second cleavage. Two normal asters are united by a spindle which is bent slightly toward the first

cleavage plane. The chromosomes are arranged in the equatorial plate. In the cytoplasm, toward the first cleavage plane, occur several chromosomes which are attached to astral fibers extending from one of the poles of the spindle. These might be interpreted as chromosomes which did not reach the nucleus during the first cleavage, but which have persisted in the cytoplasm. Frequent observation of the same phenomenon in other hybrid material has led me to think that this elimination took place during the prophase of this mitosis. In a normal prophase the elongating astral fibers push the chromosomes before them and when the asters have attained their normal distance from each other the equatorial plate is established. The abnormality shown in figure 25 consists in the extra growth of some of the astral fibers of one or both asters toward the previous cleavage plane. These fibers are always attached to chromatin and, in consequence, they stain deeply, as do the spindle fibers. The depth of the stain depends upon the number of fibers and this, in turn, upon the amount of chromatin extruded. In the cross between Stenotomus chrysops and Ctenolabrus adspersus to be described later, I have found the same process of elimination occurring. In a few instances the amount of chromatin expelled is very slight and only one or two astral fibers are involved. These fibers, although staining more definitely than the astral fibers to which no chromatin is attached, do not form as striking a picture as when the disturbance is greater. The unusual convexity of the spindle in the direction of the astral outgrowth suggests a lowering of the surface tension in the region of the first cleavage plane.

Figures 20 and 21 are the two cells of one egg. Figures 24 and 25 show the condition found in another egg of the same lot. In the egg from which drawings 22 and 23 were made no trace of such an occurrence is to be found.

Without more evidence these facts cannot be made the basis of a conclusive interpretation, but I think they indicate strongly that an elimination of chromosomes does occur in some of the individuals of this cross. It is also evident that the abnormalities in division, both lagging and elimination, are not regular in their occurrence.

No evidence of paternal inheritance in this cross has been observed. Only one individual capable of hatching has been reported, but development to an advanced stage is common. Since hatching occurs so rarely, one would hardly expect in a study of fixed material to find the conditions which precede perfect development predominating, and it is probable that all of the conditions so far described are to be correlated with the pathological characters of those defective embryos which show only maternal inheritance. That retardation during cleavage is correlated with the delayed division of some of the chromosomes is quite certain. Newman ('15, '17) emphasizes the importance of retardation in development as a factor in the formation of pathological embryos. The conditions of lagging, the time at which it first appears, and the extent to which it involves the nuclear material may be as variable as the pathological characters which it entails.

Ctenolabrus adspersus ♀ × *Fundulus heteroclitus* ♂

In view of the facts observed in Fundulus eggs fertilized by the sperm of Ctenolabrus, which produce larvae showing only maternal characters, it seemed of interest to examine the reciprocal cross in which development is less successful, in that none of the eggs survive gastrulation. I have made this cross six times with practically the same results. Usually about 75 per cent of the eggs were fertilized, although the ratio of eggs which cleaved regularly to those that cleaved irregularly because of polyspermy varied.

Cleavage resulted in the formation of a blastoderm which extended well over one side of the egg, but there was no evidence of a germ ring. In one experiment in which the eggs were examined eighteen hours after fertilization, the blastoderms had a diameter almost equal to that of the yolk, and all showed an opaque spot at one side which suggested that the region where gastrulation began had been the first to succumb. Figure 26 shows a section through a blastoderm of a hybrid which was fixed fifteen hours and forty-five minutes after fertilization.

There is no indication that the formative processes that usually accompany cleavage have operated here. Figure 27 is from a section through a Ctenolabrus embryo of the pure breed, which was fixed thirteen hours and forty minutes after fertilization. Although this embryo is younger than that shown in figure 26, the cells show the characteristic arrangement which precedes gastrulation. A comparison of the nuclei of these stages will be made later.

All of the material of the first cleavage stages obtained was preserved in Perenyi's fluid and not enough of it was examined to warrant any positive statements concerning the mitotic behavior. Well-fixed material of second cleavage stages are, however, fairly abundant and large numbers of eggs were studied.

Figure 28 shows a second cleavage metaphase spindle in three sections. The chromosomes are clearly recognizable as elongated rods of various lengths, some of them already attached at one end to fibers at the equator of the spindle. This stage is comparable to that of figures 16 and 17 of Moenkhaus's paper in which he states that division has already begun. The extension, however, of the ends of the longer rods toward the poles in his figures, as in this, does not indicate division, for, as figure 28 shows, the spindle fibers are attached at the end of the rod lying nearest the equator of the spindle or the proximal end, while the distal ends which have not yet been drawn into the equatorial plane and which sometimes project toward the poles are free from spindle fiber attachments.

Figures 29 and 30 are typical views of second cleavage anaphases. The longest rods can be identified with certainty as Fundulus chromosomes. They show no abnormality of form or behavior. In favorable sections of early anaphases the separate halves of the chromosomes appear paired, and it is very evident that normal division of the metaphase rods has occurred. The separating ranks are fairly straight.

Figures 32 and 33 show typical anaphases of the fourth cleavage. They resemble in their details the earlier cleavage figures. There is no perceptible diminution in size of the Fundulus chromosomes at the fourth cleavage stage. The proportionate sizes

of the chromosomes of the two species is maintained throughout the first four cleavages. Any change in size is relative and affects alike the native and the foreign chromatin. The synthetic processes which result in the growth of the chromosomes are evidently common to both groups, any differences that may exist being beyond the limits of ordinary observation.

On account of the larger bulk of the Fundulus chromosomes, there is here, too, an increased demand on the cytoplasm of the egg for the production of more chromatin than is required in the straight fertilized Ctenolabrus eggs. The possible effect of such a drain on the resources of the egg will be considered later.

In the great majority of cases observed, no lagging occurred. All of the anaphase stages of the reciprocal cross which were figured by Miss Morris show lagging chromosomes and form a striking contrast to the conditions found here. In this respect the crosses between Fundulus and Ctenolabrus show a difference in behavior comparable to that shown by the reciprocal crosses between Fundulus and Menidia studied by Moenkhaus. In the latter case, however, it was when Fundulus was used as the sperm parent that lagging occurred, while in the case described here, the lagging occurs when Fundulus is used as the egg parent. The degree of difference may not be as great in the Fundulus and Menidia crosses as it is in the crosses between Fundulus and Ctenolabrus, although figure 23 of Moenkhaus's paper shows marked lagging.

The crosses with Ctenolabrus clearly demonstrate that this lagging is not due to differences in the cleavage rate, as might be concluded from the crosses with Menidia. In all of the heterogeneous crosses so far studied the rate of cleavage is that characteristic of the egg species. The case under consideration is an additional and striking illustration of the same fact. The Fundulus chromosomes in the egg of Ctenolabrus divide twice as rapidly as they do in their normal environment. The effect of the Ctenolabrus egg in accelerating the division rate of the chromosomes carried in by the Fundulus spermatozoon is more impressive than the opposite retarding effect of the egg of Fundu-

lus on the spermatazoön of Ctenolabrus in the reciprocal cross in that it emphasizes the active part taken by the egg in its reaction with the spermatozoon.

In heterogeneous crosses, then, the spermatozoon exercises no influence upon the rate of cleavage of the egg. This is not true of homogeneous crosses, as both Newman and the Hertwigs have shown. In the crosses Fundulus majalis ♀ × Fundulus heteroclitus ♂ and Gobius capito ♀ × Gobius jozo ♂ development is more rapid than in either of the egg parents. The conclusion is that here the rôle of the sperm is a positive one. These facts suggest that the spermatozoon plays a more passive part in heterogeneous fertilization than when the species crossed are more closely related. They also indicate that it is the reaction between the egg cytoplasm and the spermatozoon chromosomes which causes the division of the latter and that this reaction is to be regarded as distinct from the coöperation between the chromatin elements of the conjugating nuclei which determines the hereditary character of the larva.

The fact that polyspermy is the rule in heterogeneous teleost hybridization, rather than the exception, lends weight to the argument that the ease with which the eggs of any species of fish can be fertilized by the spermatozoa of any other species is due to the general similarity in the physical organization of the teleostean germ cells and depends upon physical or chemical qualities quite distinct from the hereditary constitution of the germ substance and independent of it. In other words, the ease with which cross fertilization is carried out is not dependent upon the degree of relationship of the species crossed. The term fertilization as used here is meant to include both the process of insemination and the activities resulting in the union of the egg and sperm nuclei and their coöperation in mitosis. Polyspermic eggs afford some evidence that the participation of the paternal chromatin in mitosis in the teleost egg is due to mechanical or chemical factors of a general nature. Figure 34 shows a section through an egg which divided into three cells instead of two at the first cleavage division. There is no direct proof that this is due to polyspermy, but the combined evidence

from polyspermic eggs of this and other crosses seems to justify such a conclusion. At any rate, in this event of abnormal cytoplasmic division two quite 'normal' anaphase figures appear. These are shown in detail in figure 35 and 36. As in monospermic eggs the morphological characters of the chromosomes of the two species persist, there is no lagging on either spindle and the cleavage rate of the egg is not changed.

Occasional evidences of lagging chromosomes are met in this cross. Figure 37 represents a two-celled egg in the telophase of the second cleavage which showed lagging nuclear vesicles. Only two such instances were observed. The only other case in which lagging was observed in these early stages was on a slide among sections of normal and polyspermic eggs. The normal eggs were two-celled, the polyspermic eggs usually four-celled. The egg in which lagging occurred was apparently a two-celled egg which was in the anaphase of the second cleavage. A strand of chromatin lay stretched across the first cleavage plane and was connected by astral fibers with both spindles. That this chromatin had been left in this position at the end of the first cleavage was apparent from the fact that it had interfered with the normal extension of the cell wall which usually completely separates the first two blastomeres.

Another feature noted in the same egg, and met occasionally in this as well as in other crosses, may be described here. In the third cell of this egg two asters are present. Whether these previously formed a spindle is uncertain. The cytoplasm in the region between them has a coarser granular appearance and stains more deeply than the same region in normal Ctenolabrus eggs. If chromatin were present here it has been dissolved, but the cause of such an occurrence is still obscure. Figures 38 and 39 show the spindles from a two-celled hybrid egg in which the same granular condition of the cytoplasm in the equatorial region appears. All of the chromosomes present in the egg are drawn in these figures. It is evident that some are missing and, since there is a decided lack of hook-shaped chromosomes and a predominance of long Fundulus elements, the obvious conclusion is that some of the chromosomes of the egg are missing. This

suggests that the abnormal granular condition which occurs irregularly in these hybrid eggs is caused by the dissolution of the chromatin. Figure 40 shows one cell of a three-celled egg in which the evidence points to the same conclusion. This egg was included in a lot of eggs fixed in the eight-celled stage. There is here evidence of lagging or eliminated chromatin. There is also the same abnormally granular cytoplasm present in the region of the spindle. Some chromatin masses are present, but their outlines are indefinite. They are evidently in the process of dissolution. These, as well as the lagging chromosomes, are regarded as remnants of the first cleavage which, in this case, was so irregular that further cleavage was impossible, an interpretation which an examination of the remaining cells of this egg seems to verify. One sister cell shows an anaphase spindle with no recognizable Fundulus chromosomes, the third cell contains three asters separated by masses of granular protoplasm and lacking anything that can be identified as chromatin. In this case the dissolution of chromatin is clearly associated with a cessation of development, and it is highly improbable that any of the eggs in which extensive degenerative changes of this nature occur in the early stages survive. It would be of interest to know whether normal eggs, when fertilized in the laboratory, are ever subject to disturbances of the same kind or whether the effect is a result of hybridization. Its significance in this connection lies in the possibility that it may occur at any time during development and in varying degrees, and that in later stages, after cleavage has proceeded for some time, its effect might not be so widespread and therefore less disastrous.

The later cleavages were studied in eggs belonging to the same lot as that shown in figure 26. These had developed for nearly sixteen hours. The conditions in them varied, some eggs showing cells more uniform in size, arrangement, and nuclear character than others. Figure 41 is from a hybrid blastoderm in which seventeen mitotic figures were counted. No abnormalities were noted in any of them. In a thirteen-hour egg of the pure breed twenty-five normal anaphase figures were found. Figure 42 shows several of these, from which it is evident that the polar

masses of chromatin in the hybrid eggs show no more variability in size than do those in the straight fertilized eggs of Ctenolabrus. They present no evidence of the elimination of chromatin. One hesitates, however, to draw any conclusions from such figures, in view of the fact that the eggs differ slightly in age and, further, we have no real assurance that the relative size of these masses indicates the relative amounts of chromatin contained. They are given here to show that the possibility of obtaining evidence from this source was not overlooked.

Günther and Paula Hertwig considered that the lobed character of the nuclei in their Crenilabrus ♀ × Gobius ♂ embryos was a sign of nuclear degeneration. Figure 43 gives a series of typical nuclei from the hybrid blastoderm, of which figure 26 is a section. Nuclei from the slightly younger stage of a purely bred embryo are shown in figure 44. The similarity obtaining between these, with regard to the lobed condition, militates against the view that the lobes appearing in these older nuclei are in any sense a sign of degeneration.

The nuclei do, however, differ strikingly in one regard, and that concerns the occurrence of nucleoli. There are typically two nucleoli present in the nuclei of the normal Ctenolabrus blastoderms (fig. 45). The nuclei of the hybrid blastoderms contain typically only one nucleolus. In the hybrid blastoderms which show by their uniformity in cell and nuclear size that cell division has been fairly normal, I have found but one exception to this (fig. 43). Exceptions occur in those blastoderms in which cell division has been very irregular. Such eggs show great variation in the size of both cells and nuclei, and abnormal mitotic figures occur frequently. Variations in the occurrence of nucleoli in these eggs is to be expected. Many of the nuclei are very small and contain no nucleoli. A few nuclei show two nucleoli and in one large nucleolus three nucleoli were found. Even here single nucleoli are present in the majority of cells. There is good reason to think that this difference in the hybrid nuclei is due to the presence of the foreign chromatin.

Moenkhaus, studying the nucleoli in his fish hybrids, found that there were typically two present in each nucleus and he com-

pares his observations with those of Häcker ('95). The latter investigator found in the cells of some species of Cyclops at certain stages two nucleoli which, according to his interpretation, represent the two parental chromatin masses. Since in Cyclops the pronuclei do not fuse during fertilization and are closely apposed, but structurally separate vesicles, as late as the two-celled stage, and since the parental chromosomes remain bilaterally distributed, Moenkhaus accepts Häcker's view, but hesitates to apply the same interpretation to the double nucleoli in fish hybrids, and bases his objection upon the fact that in his hybrids the pronuclei fuse during fertilization and that the chromosomes do not remain distributed bilaterally upon the spindle, but are mingled indiscriminately at the end of the first few cleavages. Miss Morris's observations are in agreement with those of Moenkhaus as regards the mingling of the two parental groups of chromosomes in the course of cleavage, but she contends that a fusion of the germ nuclei does not occur. I have also observed that there is a more marked grouping of the two parental chromosome complexes in the second cleavage stages of the reciprocal crosses between Fundulus and Ctenolabrus than occurs in the fourth cleavage.

However, the question as to whether in these double nucleoli found in fishes we are dealing with parental homologues, is not dependent upon the proof or disproof of the fact that the parental chromatins remain segregated in the nucleus. If we are ready to grant that the chromosomes maintain their individuality throughout unlimited cell generations in other forms where there is no hope of tracing any part of the chromatin through even one cell generation, we cannot find objection to the idea that structures as closely related to the chromosomes as are these chromatin nucleoli can reappear in connection with their respective spiremes at certain phases of the nucleus. While perhaps the facts observed by Moenkhaus do not argue directly in favor of such a view, they cannot be considered as having any weighty bearing of opposite significance.

The facts presented here form strong evidence for the view that these double nucleoli are correlated with the biparental

origin of the nucleus. In the hybrid blastoderms the paternal nucleolus is lacking. Reference has been made above to the demand upon these hybrid eggs for a larger production of chromatin than is necessary in the normal Ctenolabrus eggs. The idea was advanced that this increased production was due to the presence in the egg of larger chromosomes than it normally contains and that this synthesis of extra chromatin leads to an early exhaustion of the necessary substances, so that in later stages the foreign chromatin is not able to function normally. The single nucleolus present in the hybrid egg at a stage when in normal eggs there are two may be regarded as an indication of the failure of the paternal chromatin to continue development.

I wish to point out the possible correlation between the binucleolate condition and the greater viability of the Fundulus and Menidia hybrids in contrast with the mononucleolate condition and the failure to survive gastrulation of the Ctenolabrus ♀ × Fundulus ♂ hybrids. Further study of hybrid nucleoli is necessary to decide whether or not such a correlation is of universal application.

Ctenolabrus adspersus × ♀ *Stenotomus chrysops* ♂

No direct evidence as to the number and form of the chromosomes of Stenotomus was obtained. It is inferred, however, from a study of the egg of Ctenolabrus fertilized with the sperm of Stenotomus that the chromosomal complex of the latter species closely resembles that of Ctenolabrus. Figure 50 represents an anaphase spindle of the first cleavage mitosis in this hybrid. It is very similar to figures 9 and 10 which show anaphases from pure Ctenolabrus eggs. Since the experiments yielding this hybrid material were well controlled, there can be no question of its authenticity, although the chromosomes contributed by the two parents cannot be distinguished morphologically. Similarly, an egg of Stenotomus fertilized with the sperm of Fundulus heteroclitus should show very much the same sort of chromosomes as the egg of Ctenolabrus when crossed with Fundulus, figures of which are referred to in the previous section. Figures 55 and

56 are anaphase spindles from the former cross. Although division became very irregular in this experiment and a spindle with the full quota of chromosomes is rare, nevertheless two types of chromosomes characteristic of Ctenolabrus, rods and hooks, appear. None of the typical long Fundulus chromosomes are present in figure 56 and only one appears in figure 55. Some of the short rods shown may have originated from the spermatozoon, but the hook-shaped elements are undeniably chromosomes belonging to the egg.

The cleavage rhythm of the developing eggs of Stenotomus and Ctenolabrus is approximatey the same. The hybrid eggs of the cross Ctenolabrus ♀ × Stenotomus ♂ resemble the pure Ctenolabrus eggs in the rate of the early cleavages.

No abnormalities in the behavior of the chromosomes during the early cleavages have been found. Figures 46 to 54 show in sequence the important stages studied. The union of the male and female pronuclei is shown in figure 46. Figure 47 is of a stage just after the disappearance of nuclear walls and before the chromosomes have entered the spindle. In figure 48 a slight advance is shown. The chromosomes lie on the spindle between the asters, but are not yet drawn into the equatorial plate. In both of these figures two rather well-defined groups of chromosomes appear. In the formation of the equatorial plate this grouping is lost and is no longer evident in the ensuing anaphases, as may be noted in figures 50 and 51. These figures show, also, the form of the elements of the hybrid complex and the absence of any interference in their division. Figure 49, of a polyspermic egg, is given because it shows very well the early separation of the chromosomes, a stage not observed in the monospermic eggs. The unimpeded splitting of the chromosomes is clearly demonstrated. Postanaphase stages of the second cleavage likewise reveal nothing abnormal in the nuclear behavior.

Figure 52 is a drawing of the fourth cleavage stage in which pairs of chromosomes can be identified. Not all of the chromosomes could be included in the drawing.

The occurrence of numerous vacuoles in the cytoplasm of the eggs of this lot is the only abnormality noted, but whether this

is a constant feature of this cross is uncertain. Another lot of eggs of the same cross, killed in the four-celled stage, shows very few vacuoles. At a much later stage, in still another lot of eggs, only occasional vacuoles appear.

Figures 53 is drawn from a section through a later hybrid blastoderm and shows the latest stage offered by the material studied. The mitotic figures are quite normal. Figure 54 is a polar view of an anaphase plate found in an egg of this stage. Forty-two chromosomes are present.

Some abnormal mitotic figures were present in eggs which showed very irregular cleavage and which were probably polyspermic eggs. A few instances were noted in which solid black deposits of the same nature as that shown at *A*, figure 53, but much more extensive, were present at the periphery of the egg. This deposit sometimes fills an entire cell. In some cells it occupies the region of the aster and occasionally a lagging mitosis is found near the affected region. In this case the abnormal cell division is caused by the peripheral disturbance, the nature of which is not understood.

A large number of the eggs of this experiment developed normally up to the time of hatching, but none were able to hatch. Superficially they resembled pure Ctenolabrus embryos. Newman has obtained many hatching embryos of the maternal type from this cross.

Stenotomus chrysops ♀ × *Ctenolabrus adspersus* ♂

While the cross just described is one of the most successful of the heterogeneous crosses thus far made, in that it has been carried through to hatching, the reciprocal cross, Stenotomus chrysops ♀ × Ctenolabrus adspersus ♂, has never been reared beyond the gastrulation stages. For that reason the abnormalities found in the behavior of the chromosomes in this cross are especially significant. The material studied was obtained from three experiments, and the same irregularities are found in all lots.

In this cross, as in the previous one, the only evidence that the chromosomes appearing in the cleavage mitoses comprise the

original contribution of the egg and sperm is the fact that during the early metaphase two groups of chromosomes can be distinguished on the spindle. Very often one of the groups is massed together in such a way that single chromosomes cannot be recognized, while in the other group the elements are well separated. Figure 58 shows the characteristic arrangement of the two clusters of one spindle. This may be considered rather important as an indication of physiological differences which are the cause of subsequent abnormalities.

A large number of dividing cells of the first three cleavages were studied. These differ strikingly from those of the reciprocal cross. All show lagging chromatin, some to a very marked degree. Figures 59 to 62 present four first-cleavage anaphases.

Figure 59 includes three sections arranged in the order that they appeared on the slide. Many of the chromosomes on the spindle are dividing normally. The plane of the sections probably corresponds with that of the section in figure 58. In figure 61 undivided chromosomes are passing to the poles. This forms indisputable evidence of the unequal distribution in this hybrid of chromosomes during cleavage. It is only in anaphase stages that such an abnormal occurrence could be detected.

Figures 67 and 68 are of anaphases of the second cleavage and figures 70 and 71 illustrate the same stages from the third cleavage. These figures, as well as many others in the material, show a condition which suggests that some of the chromosomes have undergone a process of fragmentation. Small round bodies, which behave like chromosomes, appear frequently in these stages. They have been observed only occasionally in the normal Ctenolabrus eggs and in the reverse cross between Ctenolabrus ♀ and Stenotomus ♂. However, the conditions here, where all of the chromosomes are scattered over the spindle, are more favorable for a study of the true form of the chromosomes than in normal mitoses, where the chromosomes moved in close ranks to the poles, and it may be that these small elements are of normal occurrence, but have hitherto escaped notice. I am inclined to favor the idea of their fragmentary nature.

Figure 67 confirms this interpretation. One of the V-shaped elements, which is the contribution of the foreign spermatozoon, shows a new and interesting adddition in the form of a round granule, a chromosome possibly, attached to one of its arms. This granule evidently became united to the V-chromosomes before division took place, because both of the mates resulting from division show the identical arrangement. I have tried to find the same combination in other cells, but although the V's occur, they are not associated with any fragments of chromosomes.

One cell was found in which division had reached a later anaphase stage. Among the chromosomes lagging at the equator of the spindle was one with subterminal fiber attachment. The two daughter halves had almost separated. The portions which extended toward the poles were drawn out into a thin thread, while the free end was condensed, having the appearance of a small granule. The thin strand of chromatin uniting these proximal ends with the distal undivided portion lying in the equatorial plane were almost ready to break. If such a break occurred, it might result in the elimination of a portion of a chromosome. Unfortunately, the reading for this section was lost and a drawing could not be made. Figure 71 shows one section of an anaphase of the third cleavage. The chromosomes are well separated and in most cases very obviously paired. In the figure a light line unites the daughter halves of one chromosome. It will be seen that some bodies cannot be thus paired. In one instance the apparent mate of a short rod consists of two small round chromosomes, probably separated chromomeres. In another the daughter halves of one chromosome still united, are passing together to one pole. They have no counterpart in the other half of the spindle.

What becomes of chromosomes which may not divide during mitosis? The evidence on this point is not abundant. Figures 63, 65, 66, and 69, which are of some of the conditions found, demonstrate clearly that not all of the chromatin reaches the poles during the anaphase or becomes incorporated in the new nucleus. Figure 63 shows chromosomal vesicles in the region of

the new cleavage plane. The cell is in the telophase. Figure 69 shows one or two chromosomes still at the equator when those at the poles have begun to form vesicles. Figures 65 and 66 show chromatin persisting in the axis of the former spindle. Figure 65 is of a prophase, viewed laterally. This may be an active extrusion of nuclear material, but the fibers which accompany the extra nuclear chromatin in this case extend to the first cleavage plane and seem to be persisting structures rather than new outgrowths from the aster. Figure 66 is from a two-celled egg in the metaphase, viewed from the poles. The figure was reconstructed from two sections. Several chromosomes lie along the fibers connecting the sister spindles. Here again the appearance resembles a structure persisting from the previous cleavage rather than one originating during the present division. The apparent interference in the completion of the dividing cell wall strengthens this impression.

The active process of elimination (during the metaphase) as described for the cross Fundulus heteroclitus ♀ × Ctenolabrus adspersus ♂ has been observed frequently in this cross. Figure 64 shows it in its less exaggerated form. This is undoubtedly one of the conditions which leads to lagging in the anaphase.

Figure 72 is of a section through the blastoderm of an egg which had developed for five and one-half hours. Such blastoderms frequently show large numbers of dividing cells, in all of which the character of mitosis is essentially similar to that of the earlier divisions. Figure 73 illustrates some of the typical abnormalities. In figure 74 is shown a section through a blastoderm in which the nuclei were in the resting stage. Here it is difficult to determine whether chromatin has been eliminated, since the eliminated chromatin does not persist in such form that it can be recognized easily. Small nucleus-like inclusions between the cells of the otherwise normal-appearing blastoderms may be the remains of chromatin which has been left in the equatorial plane. Such masses are shown in figure 75. Chromatin remaining in the cytoplasm is evidently rapidly absorbed.

A great deal of irregularity exists among the blastoderms at this period in the number of cells which they contain. While much of this irregularity is, no doubt, the direct result of early abnormalities in cleavage, some of it must be ascribed to the effects of polyspermy. No differences, great enough to be used as a definite basis for correlation with these two causes, can be seen in this material. However, we are justified, I believe, in concluding that the varying degrees of abnormality exhibited in the later stages may be correlated with the variability existing in the earlier cleavages and that both lead sooner or later to a cessation of development.

Ctenolabrus adspersus ♀ × *Menidia menidia notata* ♂

The cytological features in the Menidia-Ctenolabrus hybrids are in many respects similar to those already described in the Fundulus-Ctenolabrus crosses. Because of the close likeness of the two cases, it will be necessary only to outline briefly the conditions observed. The chromosomal complex of Menidia has been described by Moenkhaus; that of Ctenolabrus has received attention in an earlier section of this paper. From a comparison of the two descriptions it is clear that there can be no hope of recognizing the maternal and paternal chromosomes in these hybrids. As in the crosses between Stenotomus and Ctenolabrus, however, two groups of chromosomes appear on the early metaphase spindles of first-cleavage stages of the cross Ctenolabrus adspersus ♀ × Menidia menidia notata ♂. No physiological or morphological differences were observed in the two groups.

Several early cleavage stages were examined, but no abnormalities were noted. The earliest anaphase stages studied were from third-cleavage stages. In these the chromosomes divide and pass to the poles in straight ranks with almost diagrammatic regularity. Unfortunately, the sections which were made of this material were not clear enough to permit me to detect minute differences in the shapes of the individual chromosomes, and so I have not attempted to give figures of these early stages.

Figures 76 to 78 are from eggs which had developed for two hours and forty-six minutes. The mitotic figures are still of good size and the familiar rods, hooks and V's are easily made out. Since the chromosomes are crowded into less space than in the earlier cleavages, all of the elements of an anaphase plate could not be drawn with the camera. Consequently, these lateral views give no idea of the chromosomal number characteristic of the hybrids. This is shown in figure 79, which is a polar view of an anaphase plate. Forty-one chromosomes are counted.

All of the material from this cross, with, of course, the exception of polyspermic eggs, exhibits normal chromosomal behavior. In view of the character of the early cleavages, the irregularly divided blastoderms which occur frequently in the later material may be regarded as originating from polyspermic eggs. In these, multipolar mitotic figures occur. These of course show lagging chromosomes, but in the bipolar figures of the same eggs the division of the chromosomes and their migration to the poles take place in the normal manner.

Newman reports this cross among those which do not survive gastrulation. In my experiments some of the embryos remained alive for nearly three days. None hatched. All were abnormal, but no close examination was made to determine the nature of the defects.

Attention has already been called to the fact that in the cross Ctenolabrus ♀ × Fundulus ♂ the production of more chromatin by the Ctenolabrus egg is demanded than in normally fertilized eggs. This led to the idea that a possible disturbance of the metabolic processes concerned in the production of chromatin caused the early death of these eggs. In this case, Ctenolabrus ♀ × Menidia ♂, the amount of chromatin necessary for the regular development of the paternal chromosomes is not appreciably greater than in the pure Ctenolabrus egg, since the Menidia chromosomes are of about the same dimensions as those of Ctenolabrus. Their combined bulk may even be less.

Menidia menidia notata ♀ × *Ctenolabrus adspersus* ♂

One experiment in which the eggs of Menidia menidia notata were fertilized with the sperm of Ctenolabrus gave larvae which hatched. Both of these were like the pure Menidia embryos. One was healthy and one, evidently hindered by a large yolk sac, could not swim well, but lay on its back for most of the time. No attempt was made to see how long these embryos might live. Newman has listed this cross among those in which development does not go beyond the gastrulation stages, but in view of his experience in regard to the different outcome of the same cross at different times, he emphasizes his conviction that such a list can only be tentative.

The most important stages of this cross which have been studied are third-cleavage stages. Eggs in all of the mitotic phases were examined and some of the typical occurrences are seen in figures 80 to 88. In figure 80 a metaphase is shown in which nuclear material is being pushed out of the nucleus toward the inner boundary of the cell which marks the last cleavage plane. This is the same thing which was seen to occur in the cross Fundulus ♀ × Ctenolabrus ♂. It was observed only once among many eggs in the same stage, in which conditions were to all appearances normal.

Figures 81 to 85 illustrate the most frequent condition during the anaphase. In figures 81 and 82 bodies which are supposed to be undivided chromosomes are on their way to the poles. Figures 83 and 87 show respectively a mid-anaphase and a later telophase. A comparison of these figures leads to the conclusion that lagging material present at the equator in the early stages of mitosis may still be at the equator when the nuclear elements gathering at the two poles are undergoing their metamorphosis and are forming vesicles. Another telophase in which distinct chromosomes are lagging at the equator is shown in figure 88. It does not seem possible that such an abnormal delay in the division of some of the chromosomes can result in the usual precise distribution of chromosomes to the two poles.

The chromosomes show in many cases the expected forms of rods and hooks. Thickened or round bodies similar to those which Miss Morris identified as Ctenolabrus chromosomes are present, but are very inconstant in size and number (figs. 83 to 85).

In these eggs no trace of the lagging chromatin is to be found when the cells are in the resting stage. The eggs of Menidia, however, also present the same difficulties in recognizing either condensed chromatin or such chromosomal vesicles as occur in Fundulus. Any chromatin eliminated from the nucleus is probably quickly dissolved and absorbed by the egg.

GENERAL DISCUSSION

A general statement of the developmental and cytological results observed in the six heterogeneous crosses with which this study is chiefly concerned is given in table 1.

TABLE 1

CROSS	DEVELOPMENTAL RESULTS	CHROMOSOMAL BEHAVIOR
Ctenolabrus ♀ × Fundulus ♂...	Development ceases during gastrulation.	Early mitotic behavior is prevailingly normal.
Ctenolabrus ♀ × Stenotomus ♂...	Many hatching embryos of the maternal type.	Early mitoses are normal.
Ctenolabrus ♀ × Menidia ♂.....	Advanced development.	Early mitoses are normal.
Ctenolabrus ♂ × Fundulus ♀.....	One hatching embryo reported. Many advanced embryos —maternal type.	Abnormal nuclear behavior occurs.
Ctenolabrus ♂ × Stenotomus ♀..	Development ceases during gastrulation	Abnormal mitosis predominant.
Ctenolabrus ♂ × Menidia ♀.....	Two hatching embryos reported. Maternal type.	Abnormal mitosis is of frequent occurrence.

In all of the six crosses the species Ctenolabrus adspersus has been used as one of the parent species, and the list includes three reciprocal crosses. An examination of the tabulated

results reveals a number of facts that may be of value in an attempt to explain the results of hybridization on the basis of cytological processes.

1. Concerning the factors controlling mitosis

The egg of Ctenolabrus coöperates normally with the spermatozoon of three different species during the early cleavage divisions. Newman recognized the superiority of the hybridizing powers of the eggs of certain species over those of other species. From his experiments he concludes that "The three species which hybridize with the largest degree of success have small eggs, those of Tautogolabrus [Ctenolabrus], Stenotomus and Menidia." "Considering the rather large size of the eggs of Fundulus heteroclitus . . . these eggs hybridize with rather marked success." The results which he gives ('16) p. 556, table 2, show that he obtained hatching embryos from five crosses with Tautogolabrus (Ctenolabrus) and from three crosses with each of the species, Fundulus, Stenotomus, and Menidia. Of these three species, Fundulus gives the smallest total number of hatching larvae and Stenotomus ranks next to Ctenolabrus in the matter of producing individuals capable of hatching. Newman thinks that the eggs of these species contain yolk which is more easily digested than that in eggs of lower hybridizing quality.

Cytological observations have shown that the egg of Ctenolabrus is better adapted to coöperate in mitosis with the sperm of foreign species than are the eggs of either of the other three species used, Fundulus, Stenotomus, and Menidia.

I have already expressed the idea that the factors which determine whether or not a foreign spermatozoon shall take part normally in the mitotic processes are attributes of the egg. The fact that eggs of one species show the same behavior when fertilized by the spermatozoa of several different species; that the cleavage rhythm in hybrids is a function of the egg; that normal mitosis occurs in crosses in which development does not proceed very far; and, further, that the development of reciprocal hybrids is often very unlike, all offer strong support for this view. Further, if one consider the early appearance of abnormalities in

mitosis (in the cross, Stenotomus ♀ × Ctenolabrus ♂, abnormalities appear at the beginning of the first cleavage), it seems that the determining factor must be a quality of the cytoplasm and not a peculiarity of the yolk of the egg. This factor or combination of factors resembles in a general way those which cause the spermatozoon to enter the egg. Of the latter process Loeb ('16) says, "We reach the conclusion therefore that the specificity which allows the sperm to enter an egg is a surface effect which can be increased or diminished. . . ." As the rôle of surface tension phenomena in both fertilization and cell division has been given attention by many investigators, the special views concerning this need not be considered here. It may be pointed out, however, that, in fishes, the normal division of chromosomes in the environment of a foreign cytoplasm, as well as the penetration of an egg by a spermatozoön of a different species is the result of a chance fitness due to physical characters, such as viscosity, surface tension, permeability, structure, etc. of the combining or reacting agents. This may be more profitably discussed in connection with another topic.

The behavior of the chromatin of the spermatozoön during the cleavage of the egg, then, is independent of the degree of relationship existing between the species which are crossed.

2. The relation of normal mitotic behavior to development

Normal mitotic division in the early stages of cleavage is not closely correlated with normal development. Although we may reasonably expect normal chromatin distribution to be the necessary accompaniment to normal development, the preceding observations show clearly that the regular behavior of chromatin cannot be taken as a criterion of harmony existing between the germ plasms of the egg. The natural cycle of mitosis may take place if certain physical requirements of the chromatin of the sperm and the cytoplasm of the egg be fulfilled. It is independent of the relationship of the two species crossed. Having favorable cytoplasmic environment and compatible germ plasms one may expect successful development, but favorable cyto-

plasmic environment is not enough to bring about this result if the germ plasms are not harmonious.

3. The relation between abnormal mitotic behavior and abnormal development

In the last three crosses listed above, abnormalities in the behavior of the chromatin during cell division were common. In the three crosses, Fundulus ♀ × Ctenolabrus ♂, Menidia ♀ × Ctenolabrus ♂, and Stenotomus ♀ × Ctenolabrus ♂, there occur various amounts of lagging of the chromosomes during division. In the cross Stenotomus ♀ × Ctenolabrus ♂, several types of abnormalities in chromosomal behavior were found, including the elimination, fragmentation, and abnormal distribution of chromosomes. One might say that in general the degree of developmental abnormality in these hybrids was det rmined by the degree of abnormality occurring in the behavior of the chromatin. This leads us to suspect that the cause of the abnormal chromosomal behavior is the real cause of the irregular development.

One of the puzzling features of hybridization has been the wide range of variability displayed in the development of the individuals of one cross. In regard to this variability in fish hybrids Newman says:

> It may, however, be suggested that the differences in success may be due to physiological differences in the condition of the egg and sperm, for it is probable that in any large number of germ-cells forcibly stripped from males and females, some of each kind will be in a better condition for development than others.

There can be very little specificity in the germ cells of teleosts since cross-fertilization is so easily accomplished. Among sea-urchins a greater degree of specificity of the germinal products makes cross-fertilization more difficult. In the latter group surface conditions which permit cross-fertilization may be obtained by treating the germ cells artificially (Loeb, '16), or in many cases by merely allowing the eggs to stand in sea-water for a certain length of time, (Hertwig, '85; Tennent, '10).

The results of Cohn ('17) make clear the identity of these two methods.

The apparent absence of marked fertilizing specificity in the eggs and spermatozoa of fishes would permit fertilization under a wider range of conditions than would be possible in sea-urchins. Assuming that the entrance of the spermatozoön into the egg and the movements of the chromosomes during cell division are comparable processes to the extent that they have their cause in changes in surface tension, though varying in complexity and perhaps in degree of specificity, it is conceivable that slight variations in the condition of eggs and sperm which were no hindrance to fertilization might cause a disturbance in the more complex or more specific mechanism of mitosis. This would account for both individual variations within one cross as well as for the different results obtained at different times in the same cross. Examples of the latter are familiar to all who have worked with fish hybrids.

An illustration of how slight differences in the physical condition of germ cells may cause widely different disturbances in development is seen in the varying degrees of extrusion of chromatin during the early metaphase. It was suggested that a lowering of the surface tension along the newly formed cell wall was responsible for the effect, the amount of chromatin extruded depending upon the extent to which the surface tension was affected. It is reasonable to suppose that variations in the amount and in the identity of the chromatin expelled from the nucleus under such conditions would be followed by extremely diverse results in the nuclear constitution and consequently in the fate of the developing egg.

The work of Gray ('13) presents some observations which are of interest in connection with the hypothesis that the character of mitosis is a function of the cytoplasm of the egg. By treating normally fertilized eggs of Echinus acutus, one hour after fertilization, with hypertonic salt solutions he was able to cause an elimination of chromatin similar to that obtained by cross-fertilizing eggs of the same species with the sperm of Echinus esculentus. Because this treatment causes changes in

the permeability of the egg, Gray puts forward the hypothesis that the effects of hybridization in sea-urchins are due to changes in the permeability of the egg, which differ according to the species of sperm that is used. The crosses with fishes do not bear out the idea that the effects of hybridization are a function of the spermatozoa, for in three crosses with the same egg and different spermatozoa the same result was obtained, namely, normal nuclear division; while in three crosses in which the same species of spermatozoa was used to fertilize three different species of eggs, the outcome, though not the same in each of the three crosses, was similar in that abnormal behavior of the chromatin was noted in each. The greatest interest for us here lies in the fact that artificial treatment of the eggs can modify the character of mitosis, demonstrating that it is the condition of the cytoplasm that governs the nuclear behavior and not the interacting germ plasms, for in this case the two parental germ plasms are the same.

4. The respective rôles of the germ nœclei and the cytoplasm of the egg in fish hybrids

In spite of the fact that a great deal of variation occurs in the individuals obtained from one cross, rather broad differences in the developmental success of the various crosses may be recognized, and from table 1 a curious relation existing between the crosses listed there may be noted. In the cross in which nuclear behavior is abnormal the greatest success in development occurs in the more distantly related species, while, in cases in which mitotic behavior is normal, the converse is true, the highest success in development is obtained in crosses between the more closely related species.

The cases in which the behavior of the chromatin has been studied are as yet almost too few to warrant a general application of this statement, but the idea is one that will bear testing. It is reasonable to think that the results of a cross will be more nearly an expression of the relationship between the two interacting germ plasms if the cytoplasm of the egg furnishes no hin-

drance to development. This is the case in the crosses with Ctenolabrus cited here.

On the other hand, if the cytoplasm be unfavorable to the foreign spermatozoon as we conclude when early mitosis is frequently abnormal, the foreign spermatozoon will not be able to exercise its full effect against the egg nucleus. If the cytoplasm of the egg succeed in suppressing the influence of the spermatozoon entirely, we may obtain normal embryos of the maternal type. Thus we might have essentially parthenogenetic development in the sense in which Loeb claims ('12), with the participation in mitosis of the paternal chromatin, if the hypothesis advanced above is true, that in fishes, the participation in mitosis of the paternal chromatin partakes of the same nonspecific nature as does the act of impregnation itself.

There is as yet no proof that the nuclei of hybrid embryos of the maternal type contain, unchanged, the ful number of maternal and paternal chromosomes. Complete development is so rare that, without proof, we cannot exclude the possibility that a chance elimination of the right chromosomal elements—even the entire paternal complex—is responsible for it. The character of mitosis in the cross, Ctenolabrus ♀ × Stenotomus ♂ which gives many hatching embryos of the maternal type is regarded as favoring the view that perfect development is correlated with normal mitosis. It may be that in cases where abnormal mitoses prevail, very different conditions bring about the same results. However it may be, whether the chromosomes of the spermatozoon are completely eliminated or completely retained, the result, the production of a hatching embryo of the maternal type, is comparable to the parthenogenetic development of an egg. The chromosomes of the spermatozoön are without influence.

The objection might be raised here that complete recessiveness of the paternal characters does not imply a complete loss in potency of the paternal chromosomes. Newman's view on this point has been mentioned in the introductory section of this paper. If, however, the hybrids which are reared to hatching still retain both parental nuclear components, it is unlikely

that they would ever produce mature germ cells, since there would be much hindrance to a normal synapsis preceding the reduction divisions.

On this basis, the character of the recessiveness shown in heterogeneous crosses is not comparable to that displayed in some intervarietal crosses.

The occurrence of embryos showing paternal inheritance has been advanced as proof that the spermatozoon does exercise some influence upon the development of the egg. All of the paternal characters so far recognized as attaining their full development in fish embryos are characters of rather an unimportant type, such as chromotophores, which would not be expected to have much effect in the inhibition of development. It is conceivable that the factors which determine chromatophores may differ very slightly in degree in different genera as compared with factors which determine characters of a more fundamental nature —size, body form, etc.—and in so far as the interaction between the parental characters for chromatophores are concerned, we may be dealing with a phenomenon common to all fish hybrids, homogeneric as well as heterogeneric.

The appearance of paternal chromatophores indicates the retention of the paternal chromosomes. Whether the pathological features of such embryos are to be regarded as due to the interaction of paternal and maternal chromatin is uncertain. They can be accounted for by the mechanical hindrances to development shown to exist in some crosses.

In general the facts obtained from this study confirm the view of the Hertwigs as to the factors which are responsible for the results of hybridization, but they indicate that the relative importance given by the Hertwigs to these factors should be reversed. The apparently anomalous development of fish hybrids does not depend, first, upon the combination of idioplasms, and, second, upon the interaction of the idioplasm of the sperm and the cytoplasm of the egg, but, first, upon the effect of the cytoplasm on the sperm, and, second, upon the reaction between the two germ nuclei. We must add to these a third factor upon which the action of these first two depends, namely,

the variable specificity of the effect of the cytoplasm toward the foreign spermatozoön.

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EXPLANATION OF PLATES

All figures were first drawn with the aid of a camera lucida. Except when otherwise stated, the magnification of the original drawings was 1630 diameters. These were then enlarged two diameters and finished with careful reference to the object. They were reduced one-half in reproduction. Although it is difficult to draw accurately such small objects as fish chromosomes, all possible care has been taken to preserve their true proportions.

PLATE 1

EXPLANATION OF FIGURES

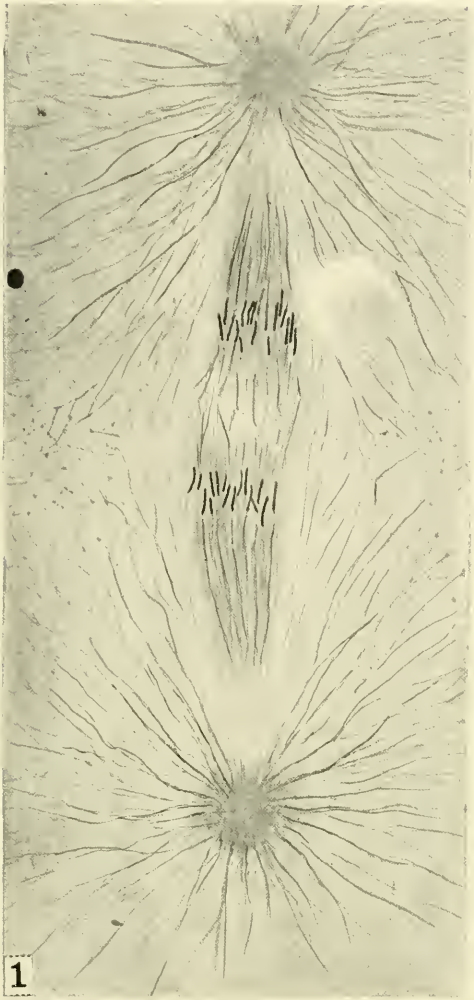
1 to 5 *Fundulus heteroclitus* ♀ × *Fundulus heteroclitus* ♂.

1 and 2 Anaphase of the third cleavage. The drawings do not show all of the chromosomes that were present in the section.

3 Polar view of an anaphase plate of the third cleavage. Forty-five chromosomes present. Magnification about 1800 diameters.

4 An early anaphase of the third cleavage showing the shorter rods.

5 An early anaphase of the third cleavage. Division is not complete in all of the rods.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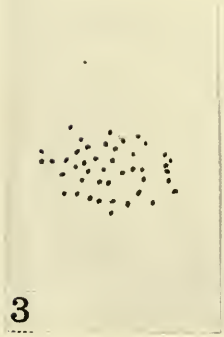
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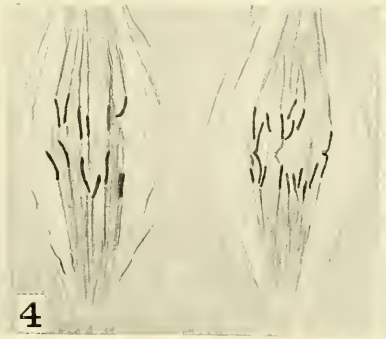
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PLATE 2

EXPLANATION OF FIGURES

6 to 12 *Ctenolabrus adspersus* ♀ × *Ctenolabrus adspersus* ♂.

6 Metaphase of the fourth cleavage in three sections. The rods are split preparatory to division.

7 An anaphase of the fourth cleavage in three sections.

8 and 10 Anaphases of the second cleavage.

9 Anaphase of the fourth cleavage.

11 Polar view of an anaphase plate from a fourth-cleavage spindle. Thirty-eight chromosomes. Magnification about 1800 diameters.

12 Same. Forty-one chromosomes. Magnification about 1800 diameters.

6

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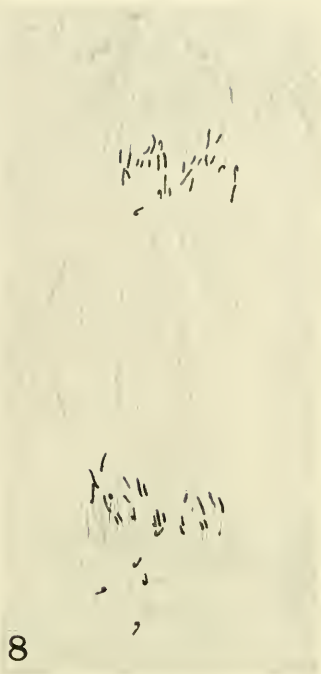

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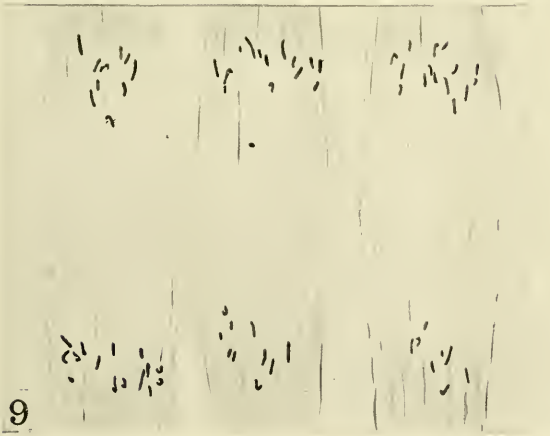

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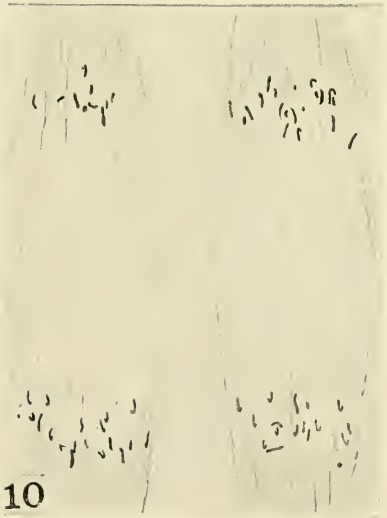

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PLATE 3

EXPLANATION OF FIGURES

13 to 17 *Ctenolabrus adspersus* ♀ × *Ctenolabrus adspersus* ♂.

13 Polar view of an anaphase plate of the fourth cleavage. Forty-four chromosomes. Magnification about 1800 diameters.

14 Polar view of an anaphase plate of the second cleavage. Forty-four chromosomes. Magnification about 1800 diameters.

15 Same. Forty-eight bodies, probably not all are entire chromosomes. Magnification about 1800 diameters.

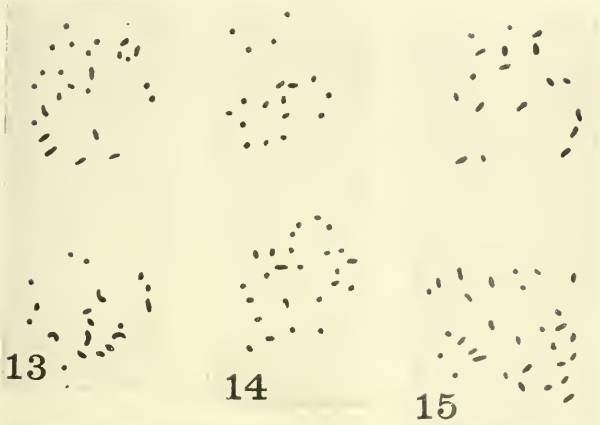
16 Telophase group of one section showing the transformation of the chromosomes into vesicles.

17 Young nucleus composed of chromosomal vesicles.

18 to 19 *Fundulus heteroclitus* ♀ × *Ctenolabrus adspersus* ♂.

18, A, B, and C. An anaphase of the second cleavage cut in three sections. The sections are oblique to the long axis of the spindle. The two poles of the spindle are lettered, respectively, *X* and *Y*.

19, A and B Two sections of a spindle of the second cleavage. *X*- and *Y* indicate the two poles.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17

X
A
Y
B
Y
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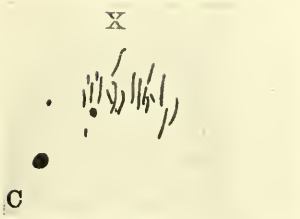
X
C

X
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PLATE 4

EXPLANATION OF FIGURES

20 to 23 *Fundulus heteroclitus* ♀ × *Ctenolabrus adspersus* ♂.

20 and 21 Both metaphase spindles of a two-celled egg shown in their correct relative positions. The elimination of chromosomes is toward the first cleavage plane in both cases.

22 and 23 Metaphase spindles from another egg of the same lot as that shown in figures 20 and 21. No elimination of chromatin from the spindle was seen in either cell. The convexity of the spindle is toward the first cleavage plane.

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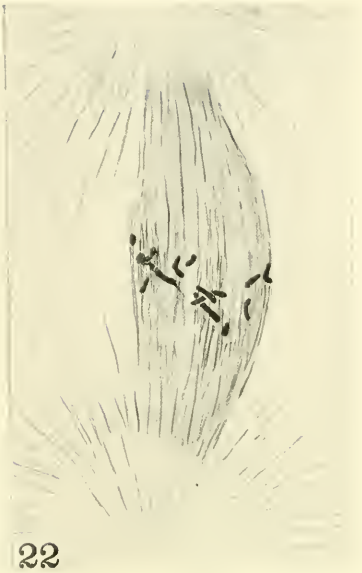
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PLATE 5

EXPLANATION OF FIGURES

24 and 25 *Fundulus heteroclitus* ♀ × *Ctenolabrus adspersus* ♂. Metaphase spindles from a two-celled egg of the same lot as those figured in plate 4. The figures are reconstructions from two sections. The elimination in each case is toward the first cleavage plane. All of the chromosomes present on the spindle do not appear in the drawing.

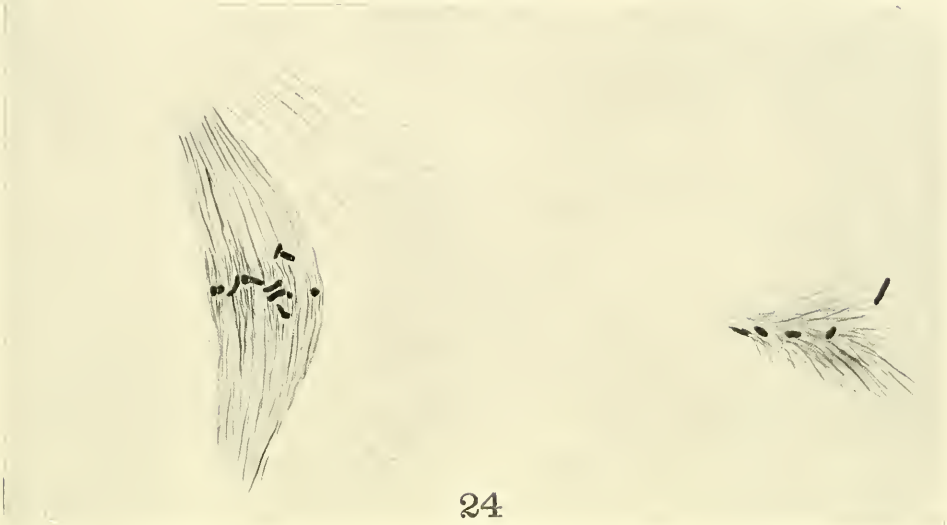

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PLATE 6

EXPLANATION OF FIGURES

26 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂. Section through a blastoderm which was killed fifteen hours and forty-five minutes after fertilization. × 170.

27 *Ctenolabrus adspersus* ♀ × *Ctenolabrus adspersus* ♂. Section through a blastoderm killed thirteen hours and forty minutes after fertilization. × 170.

28 to 33 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂.

28 A metaphase spindle of the second cleavage cut in three sections, showing a mixture of short and long rods.

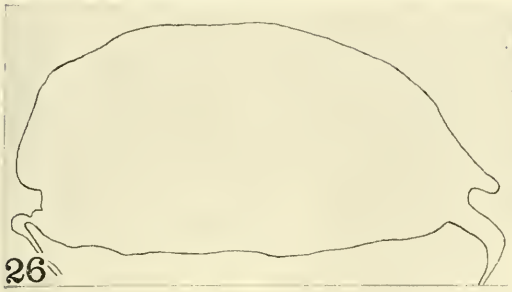
29 An anaphase of the second cleavage in two sections. The long rods characteristic of Fundulus and the shorter rods and hooks of Ctenolabrus are clearly distinguished.

30 Two sections of another spindle of the second cleavage. One section shows only the longer chromosomes contributed by the sperm. The right-hand section shows chromosomes of both parental types.

31 Polar views of the two anaphase plates of one spindle. Second cleavage. Forty-two chromosomes in each group.

32 Anaphase of the fourth cleavage in four sections. An attempt was made to draw all of the chromosomes. Only thirty-eight elements could be distinguished at either pole.

33 One section of an anaphase of the fourth cleavage. The three types of chromosomes characteristic of Ctenolabrus, rods, hooks, and a V are shown as well as the larger rods contributed by the spermatozoön of Fundulus.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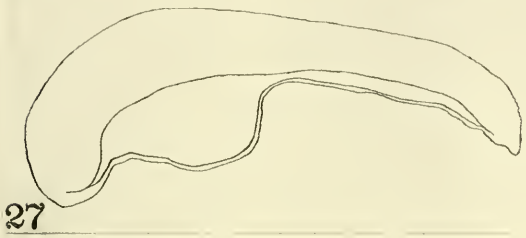
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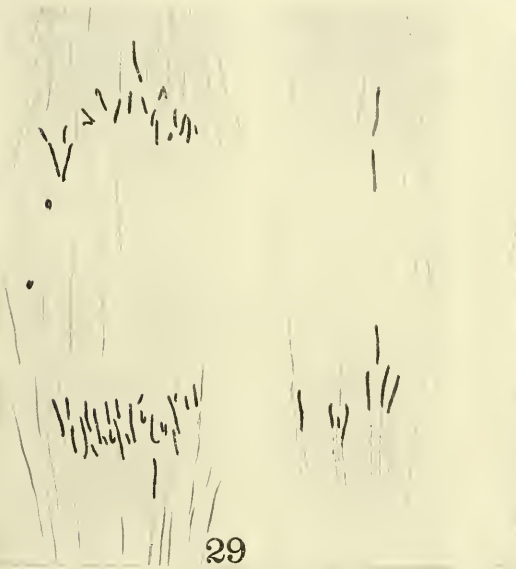
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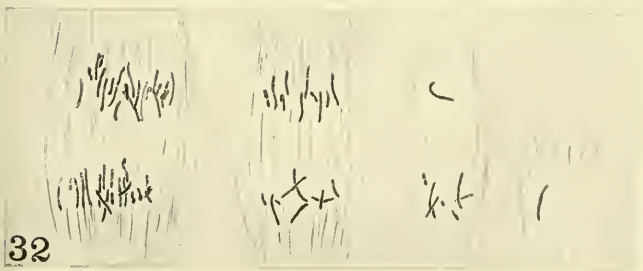
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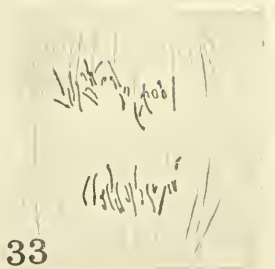
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PLATE 7

EXPLANATION OF FIGURES

34 to 39 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂.

34 A section through a polyspermic egg that cleaved at once into three cells. The cleavage walls are not complete between the cells. No chromatin was found on the spindle in the right-hand cell.

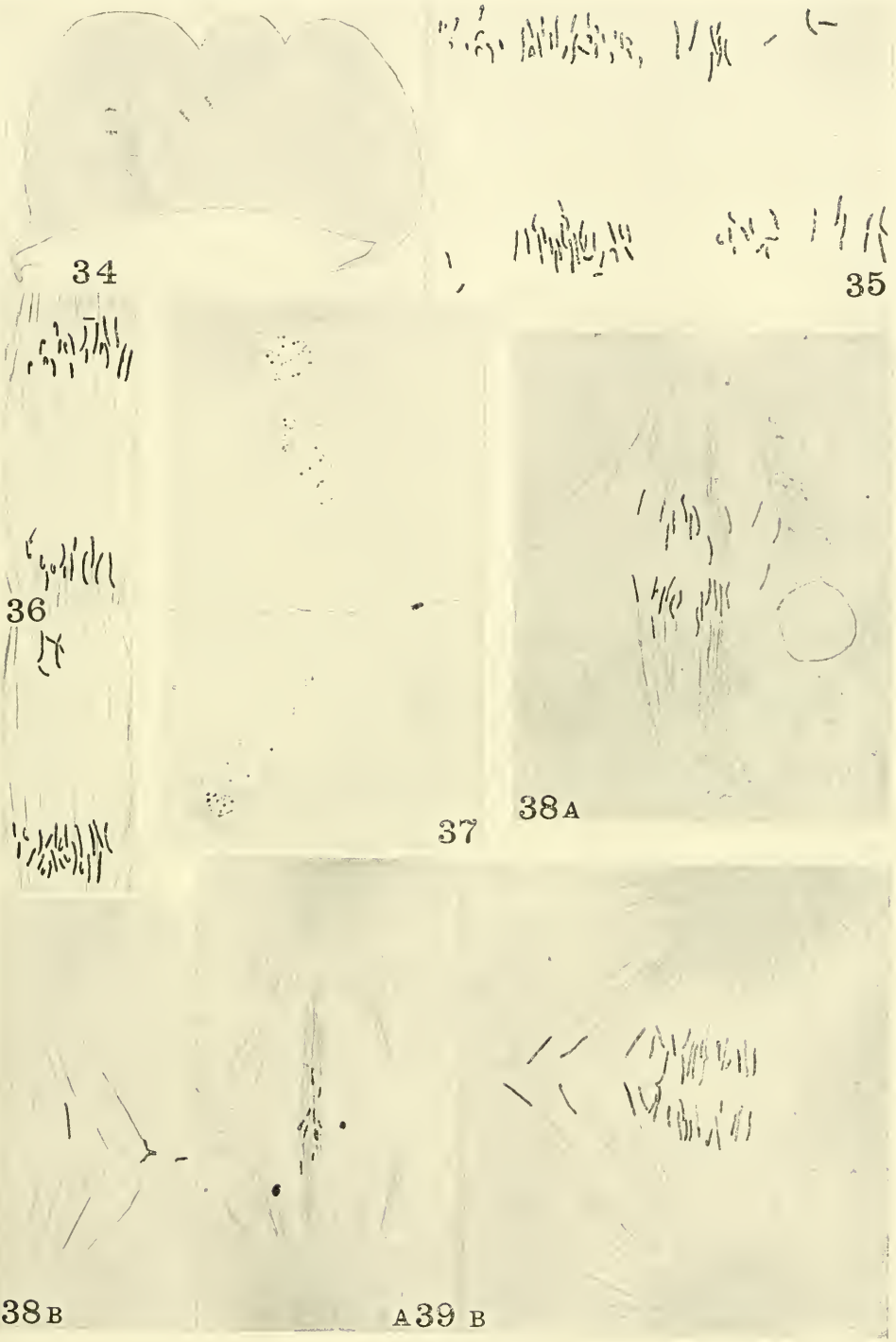
35 The anaphase chromosomes from the central cell in figure 34. The spindle was cut in four sections. Forty-two and forty-three elements are counted at the upper and lower poles (in the figure), respectively. All chromosomes drawn.

36 The anaphase chromosomes from the left-hand cell of figure 34. Not all of the chromosomes could be shown in the drawing, although they are clearly stained and well separated in the section.

37 Telophase of the second cleavage. Some chromatin vesicles extend toward the first-cleavage wall. The lagging is in the corresponding daughter cells. Magnification not recorded.

38, A and B Two sections of an early anaphase of the second cleavage. The spindle is drawn out toward the cell wall which separates the two cells. All of the chromosomes appear in the figures.

39, A and B A sister cell to the one shown in figure 38. All of the chromosomes are drawn.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38A
38B
A39 B

PLATE 8

EXPLANATION OF FIGURES

40 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂. The spindle from a three-celled polyspermic egg. The chromatin on the spindle is disintegrating.

41 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂. Mitotic figures from a sixteen-hour blastoderm. Separate chromosomes cannot be distinguished. No lagging of chromatin noted.

42 Similar figures from a thirteen-hour blastoderm of *Ctenolabrus* ♀ × *Ctenolabrus* ♂.

43 *Ctenolabrus adspersus* ♀ × *Fundulus heteroclitus* ♂. Resting nuclei from a sixteen-hour blastoderm showing the single nucleolus characteristic of regularly divided hybrid eggs.

44 Resting nuclei from a thirteen-hour blastoderm of *Ctenolabrus* ♀ × *Ctenolabrus* ♂, showing the normal binucleolate condition.

45 *Ctenolabrus* ♀ × *Ctenolabrus* ♂. Part of a section through a normal thirteen-hour blastoderm. This drawing shows the frequency with which nuclei containing two nucleoli occur in the sections. Magnification not recorded.

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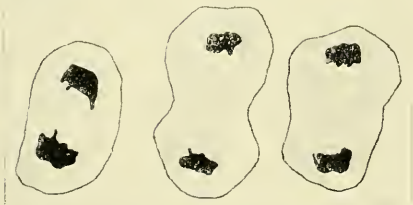

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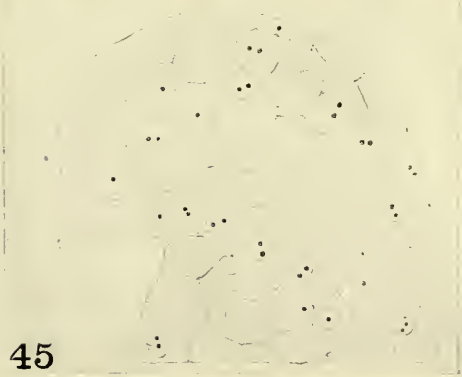
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PLATE 9

EXPLANATION OF FIGURES

46 to 51 *Ctenolabrus adspersus* ♀ × *Stenotomus chrysops* ♂.

46 The conjugating male and female pronuclei as they appeared in two neighboring sections.

47 Prophase of the first cleavage.

48 Metaphase of the first cleavage.

49 A multipolar mitosis of the first cleavage, probably due to polyspermy. The three types of chromosomes, rods, hooks, and V's, characteristic of Ctenolabrus can be distinguished.

50 and 51, A Characteristic anaphase figures of the first cleavage. No lagging chromatin.

51, B A section through six cells of an eight-celled egg from which figure 51,A was drawn, showing numerous vacuoles in the cytoplasm. (Drawn with Zeiss D objective and No. 2 ocular at table level.)

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51A

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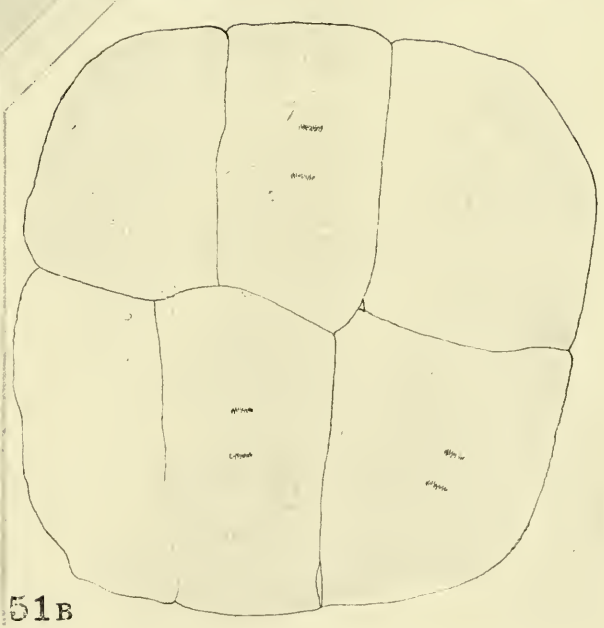

51B

PLATE 10

EXPLANATION OF FIGURES

52 to 54 *Ctenolabrus adspersus* ♀ × *Stenotomus chrysops* ♂.

52 Typical anaphase figure of the fourth cleavage in two sections. Many chromosomes omittted.

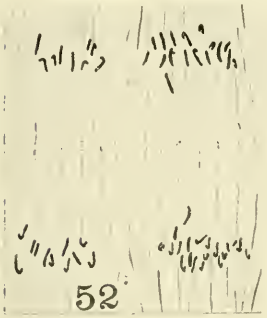
53 Part of a section through a blastoderm killed four hours and forty minutes after fertilization. No lagging chromatin was noted in any of the dividing cells. *a*, Black deposit which sometimes fills entire cells, usually peripheral cells. (Drawn at table level with No. 2 ocular and Zeiss objective D).

54 Polar view of an anaphase plate of the same stage as that from which figure 53 was drawn. Forty-two chromosomes present.

55 *Stenotomus chrysops* ♀ × *Fundulus heteroclitus* ♂. A mitotic figure from an egg which had cleaved irregularly. Chromosomes of the Ctenolabrus type are present. On the opposite side of the aster included in the figure are some chromosomes which are not taking part in mitosis although they exhibit the longitudinal cleft which precedes division.

56 *Stenotomus chrysops* ♀ × *Fundulus heteroclitus* ♂. An early anaphase. Only one section drawn.

57 *Stenotomus chrysops* ♀ × *Ctenolabrus adspersus* ♂. A section through an egg in the metaphase of the first cleavage. A—Cytoplasm. B—Yolk. Magnification not recorded.



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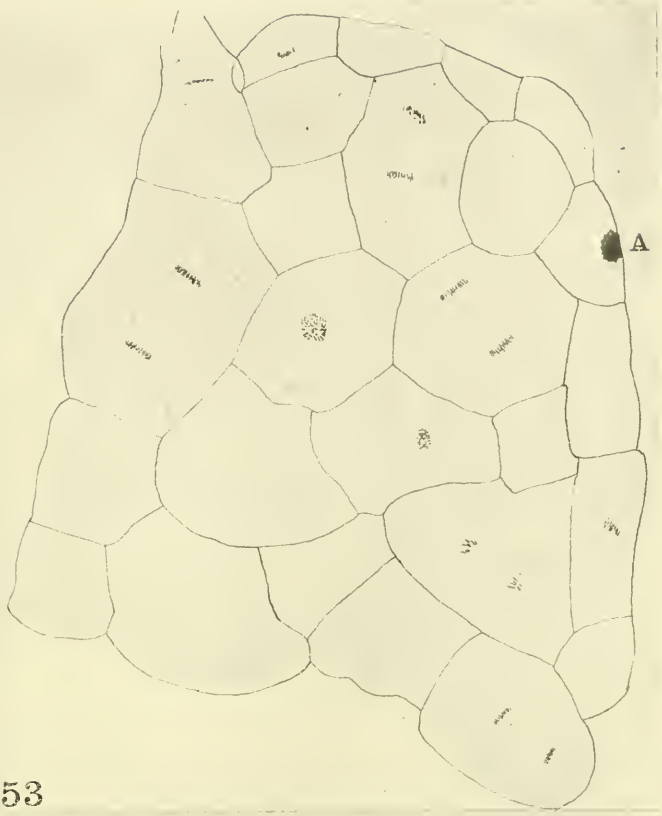

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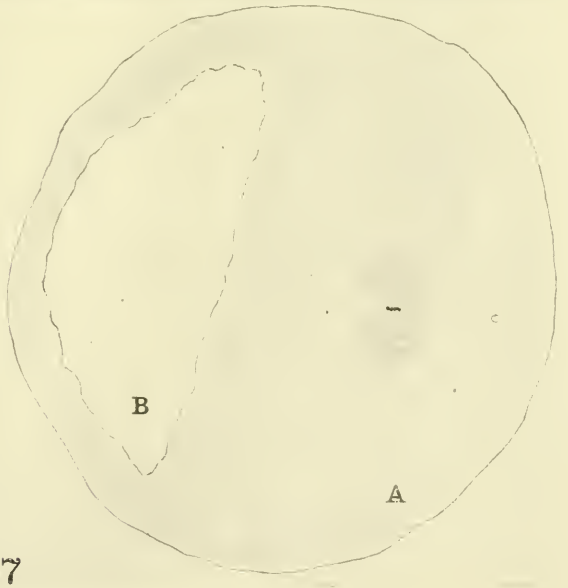

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PLATE 11

EXPLANATION OF FIGURES

58 to 63. *Stenotomus chrysops* ♀ × *Ctenolabrus adspersus* ♂.

58 Metaphase of first cleavage in two sections. In the right-hand section the chromosomes are massed together so that single elements cannot be distinguished.

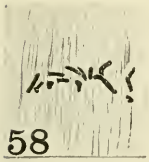
59 Anaphase of the first cleavage in three sections. The individual chromatin is situated on one side of the spindle and appears in the left-hand section of this series. The divided elements retain their characteristic form.

60 Anaphase of the first cleavage in two sections. A great deal of undivided chromatin is present at the equator of the spindle.

61 Same as 60. Only one section drawn.

62 Same as 60. Only one section drawn.

63 A section through an egg in the telephase of the first cleavage. Some chromosomal vesicles are still near the plane of cleavage. The section is oblique to the axis of the spindle and does not contain the main portions of the reforming daughter nuclei.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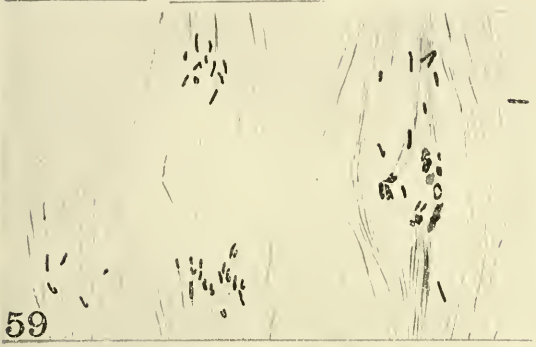
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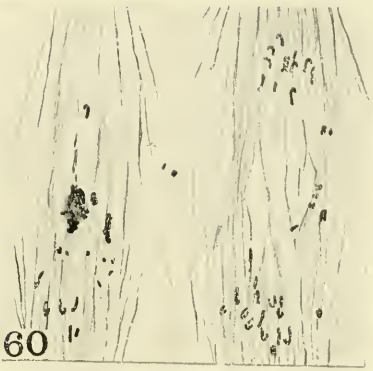
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PLATE 12

EXPLANATION OF FIGURES

64 to 69 *Stenotomus chrysops* ♀ × *Ctenolabrus adspersus* ♂.

64 Metaphase of second cleavage. Chromatin is being pushed out by the lengthening astral fibers toward the first cleavage plane.

65 Prophase of the second cleavage. The chromosomes extending toward the first cleavage plane were probably left there during the first cleavage.

66 A section through a two-celled egg in the metaphase of the second cleavage. The section passes through the equatorial region of both spindles, which are connected by fibers. Chromatin occurs along the course of the connecting fibers. Magnification is approximately 810 diameters.

67 Anaphase of the second cleavage in two sections, A and D. A is supplemented by extra drawings, B and C. B shows the elements from section a which appear in one plane. C shows on a larger scale the form of the compound chromosome described in the text.

68 Anaphase of the second cleavage in two sections, showing lagging chromatin.

69 Telophase of the second cleavage showing lagging chromosomes.

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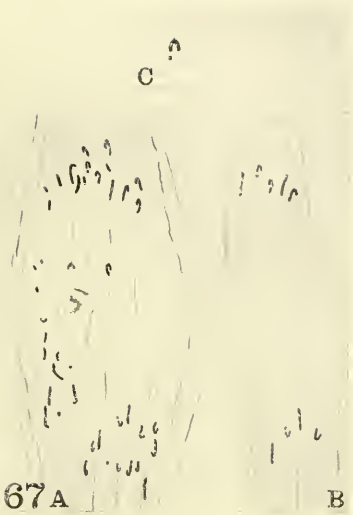

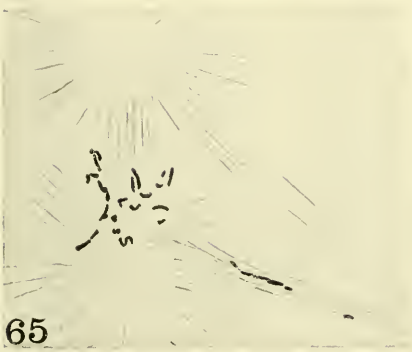

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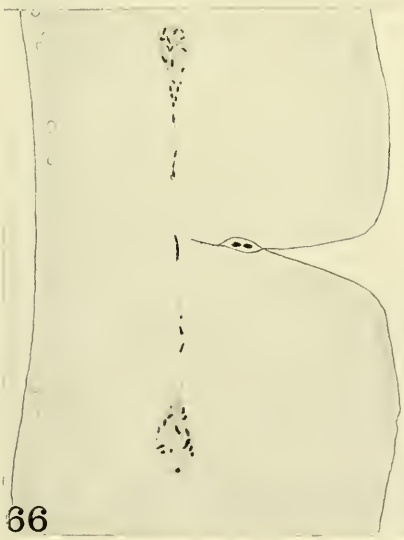

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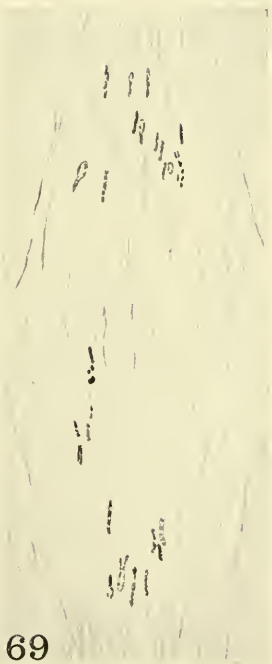

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PLATE 13

EXPLANATION OF FIGURES

70 to 75 *Stenotomus chrysops* ♀ × *Ctenolabrus adspersus* ♂.

70 Anaphase of the third cleavage in two sections. Slight lagging.

71 Anaphase of the third cleavage showing unequal distribution and fragmentation of chromosomes.

72 Section through a blastoderm killed five and one-half hours after fertilization. Magnification not recorded.

73, A and B Shows abnormal mitoses occurring in cells of blastoderms belonging to the same lot as that shown in figure 72. × 810.

74 Portion of a section through a blastoderm showing the character of the resting cells.

75 Intercellular masses which resemble nuclei more than cytoplasm in the coarser and more chromatic nature of the granulations which they contain.

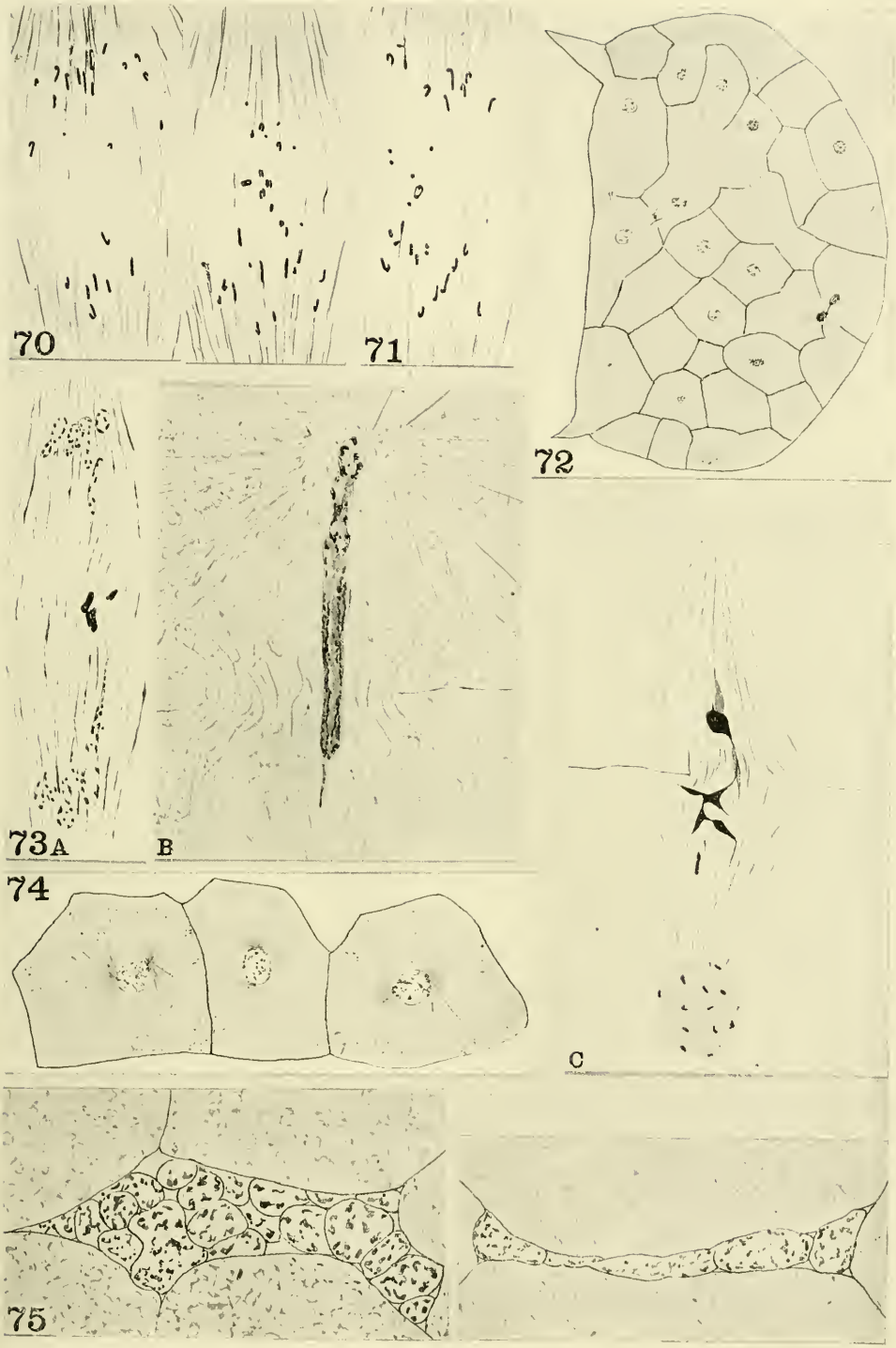
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PLATE 14

EXPLANATION OF FIGURES

76 to 79. *Ctenolabrus adspersus* ♀ × *Menidia menidia notata* ♂.

76, 77, and 78 Typical anaphases from eggs which were killed two hours and forty-six minutes after fertilization. Only one section of each spindle is drawn and not all of the chromosomes present in the sections were drawn.

79 A polar view of an anaphase plate from the same material as that used for the previous drawings. Forty-one chromosomes counted.

80 to 88. *Menidia menidia notata* ♀ × *Ctenolabrus adspersus* ♂.

80 Metaphase of fourth-cleavage. Chromatin is being pushed out toward the newly formed cell wall.

81 Anaphase of third cleavage. Entire chromosomes are passing to one of the poles.

82 Same as 81, A Undivided chromosomes.

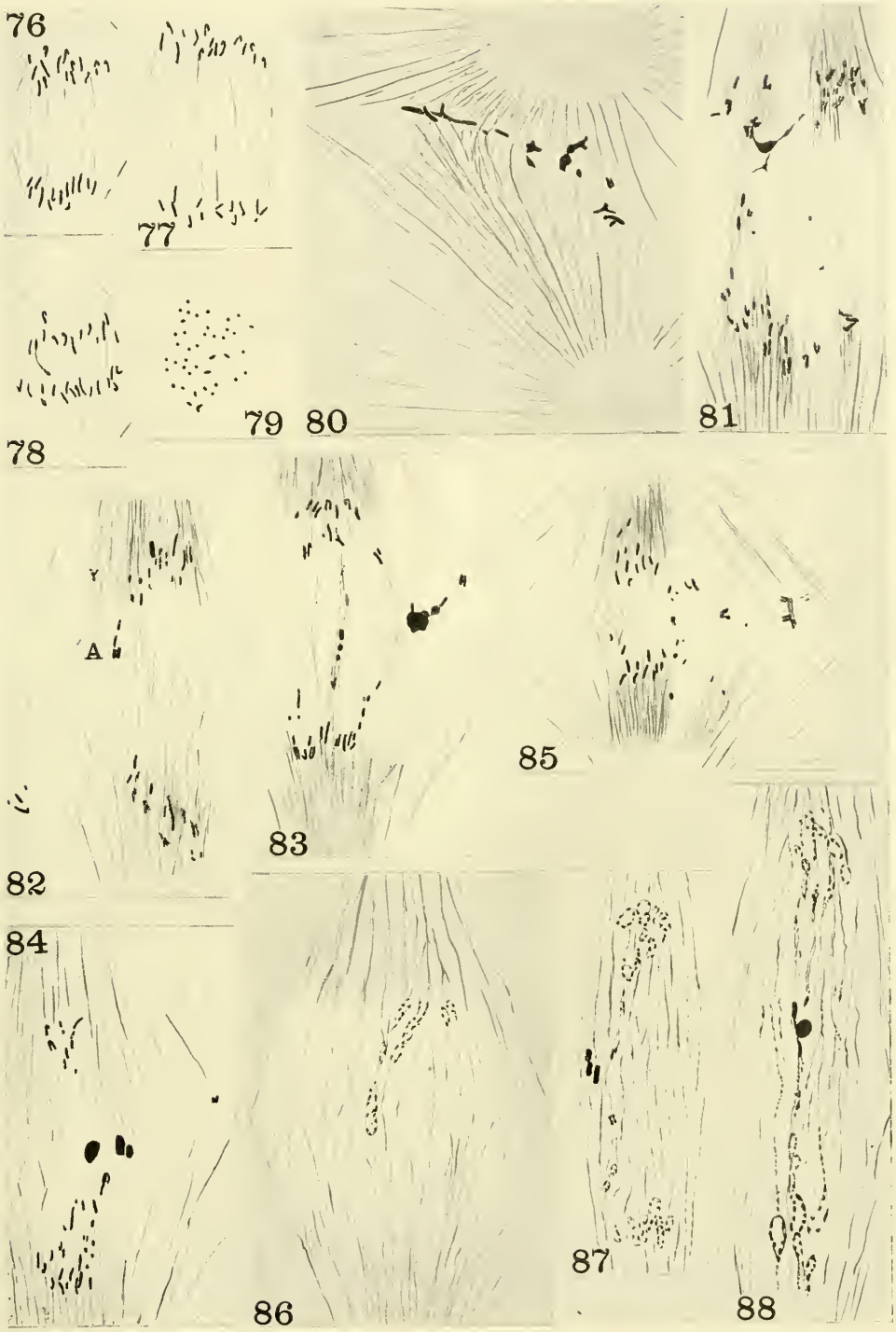
83 Anaphase of third cleavage. Chromatin masses at the equator of the spindle.

84 Same as 83. Much undivided chromatin at the equator of the spindle.

85 Anaphase of the third cleavage. Chromatin which is not undergoing division is lying at one side of the spindle.

86 Shows the lagging chromosomal vesicles extending from one pole of the spindle to the equator.

87 and 88 Telophases of the third cleavage. Both spindles show unexpanded chromatin at the equator of the spindle.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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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NEUROMERES AND METAMERES

H. V. NEAL

Department of Biology, Tufts College, Mass., and the Harpswell Laboratory, South Harpswell, Maine

SEVENTEEN TEXT-FIGURES

Two interpretations of the so-called neuromeres of vertebrate embryos have been suggested:

1. The non-phylogenetic interpretation that neuromeres are either *a*) artifacts due to the action of fixing agents or, *b*) transient embryonic structures resulting either from longitudinal compression of the neural tube or to the local strain of related nerves.

2. The phylogenetic interpretation that neuromeres are the visible remnants of a primitive segmentation of the nervous system and consequently reliable clues to the original number of metameres in the vertebrate head.

Which of these interpretations are we to accept? Are neuromeres reliable criteria of the metamerism of the vertebrate head? The present paper attempts to give an answer to these questions on the basis of the evidence presented by the central nidular relations of the motor cranial nerves, as disclosed in Bielschowsky-Paton and Cajal-Ranson preparations of Squalus embryos, and upon the data supplied by comparative embryology. The literature dealing with the neuromeric problem has been so thoroughly reviewed by Kupffer ('05, '06), Griggs ('10), Gräper ('13), and Smith ('14) that a critique and review seems superfluous at this time, and we may therefore pass at once to a discussion of the problem.

The supposition that neuromeres are artifacts produced by the action of killing and fixing fluids may be dismissed at once as erroneous on the ground that neuromeres are visible in the living embryo. That they are the purely mechanical result of the

pressure of adjacent somites is very possible—in the opinion of the writer, very probable—in the case of the neuromeres in the trunk region, where they develop in correlation with the development of the mesodermic somites and later disappear as the somites lose their rounded form and close contact with the wall of the neural tube. The hindbrain neuromeres ('rhombomeres'), however, never show a regular alternation with the mesodermic somites such as obtains in the trunk region, and, moreover, they persist in the medulla long after all traces of mesodermic somites

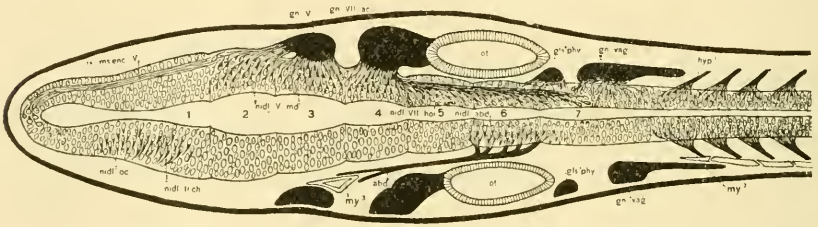


Fig. 1 A diagram of a horizontal section in the head region of a 13-mm. embryo of Squalus acanthias, showing the neuromeres and their motor nerve relations. The nidulus of the oculomotor lies in the somatic-motor column of the midbrain. That of the trochlearis is continuous with that of the oculomotor, but lies chiefly in the first (cerebellar) rhombomere. The motor fibers of the trigeminal (ramus mandibularis trigemini) are connected with neuroblasts lying within the second and third rhombomeres. The motor nidulus of the facialis is remarkable, extending through four rhombomeres (rhombomeres 4 to 7). The nidulus of the abducens extends through rhombomere 6 into the adjacent rhombomeres 5 and 7. The motor nidulus of the glossopharyngeal nerve lies partly in rhombomere 6 and partly in rhombomere 7, while that of the vagus lies posterior to the nidulus of the glossopharyngeal in a region devoid of neuromeric divisions at this stage of development. For the sake of clearness, somatic motor niduli of the cranial nerves are shown in the left wall of the brain, while the splanchnic motor niduli are shown only in the right wall.

disappear. Furthermore, the rhombomeres appear in some embryos, e.g., those of Elasmobranchs, as local paired thickenings of the wall of the medulla (Neal, '98). The rhombomeres, therefore, may not be adequately interpreted as the passive results of mechanical bending or pressure. Moreover, the fact that the differentiation of a typical rhombomere (3, figs. 1 and 2) occurs independently of connection with a cranial nerve root, proves conclusively that rhombomeres may not be interpreted

as the result of the mechanical pull of nerve roots. Therefore, however skeptical we may be regarding the morphological value of the neuromeres of the trunk region, the possibility of a phylogenetic interpretation of the rhombomeres must be granted.

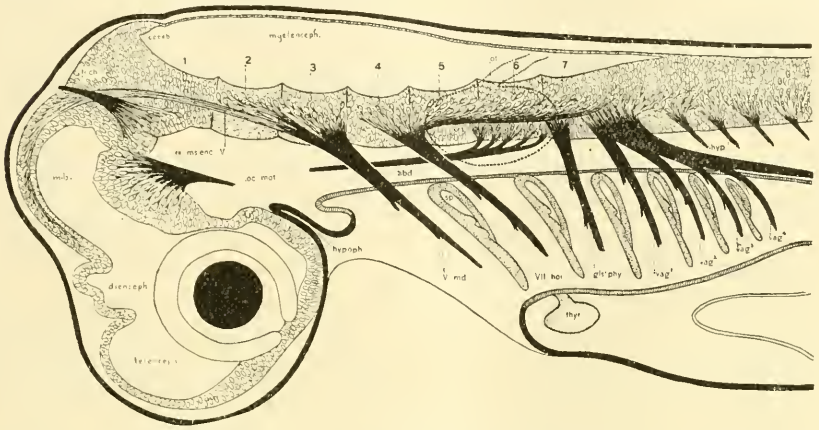


Fig. 2 A diagram based upon a parasagittal section in the head region of a Squalus embryo of 13 mm., showing the rhombomeres and their motor nerve relations. The figure supplements figure 1 in showing the distribution of the splanchnic motor nerves to the visceral arches. The origin of the facialis from four rhombomeres and its distribution to a single visceral arch is an especially noteworthy feature of the diagram. The relations shown in Squalus are identical with those described by Gräper ('13) in mammalian embryos. Abbreviations: *1.–7.*, Rhombomeres 1 to 7; *V.md.*, ramus mandibularis trigemini; *VII.hoi.*, ramus hyoideus facialis; *abd.*, abducens nerve; *cereb.*, cerebellar anlage; *dienceph.*, diencephalon; *gls'phy.*, glossopharyngeus ganglion; *gn.V.*, ganglion of trigeminus; *gn.VII.ac.*, ganglion of acustico-facilis nerve; *gn.vag.*, ganglion of the vagus; *hyp.*[1], most anterior root of the hypoglossus; *hypoph.*, hypophysis; *m–b.*, midbrain vesicle; *myelenceph.*, myelencephalon; *my.*[3], *my.*[7], myotomes 3 and 7; *nidl.abd.*, nidulus of the abducens; *nidl.oc.*, nidulus of the oculomotorius; *nidl. tr'ch.*, nidulus of the trochlearis; *nidl.V.md.*, nidulus of the ramus mandibularis trigemini; *nidl.VII.hoi.*, nidulus of the ramus hyoideus facialis; *oc'mot.*, oculomotorius; *ot.*, otic capsule; *rx.ms'ench.V.*, radix mesencephalica trigemini; *sp.*, spiracle; *telenceph.*, telencephalon; *thyr.*, thyreoid anlage; *vag.*[1], *vag.*[2], *vag.*[3], *vag.*[4], posttrematic branches of the vagus nerve.

While the majority of students of the neural segmentation incline to the opinion that neuromeres exist in the region of the forebrain and midbrain, there is so little agreement as to the essential criteria of neuromeres in this region and consequently

as to the number, that the morphological standing of neuromeres in front of the hindbrain is problematic. The future use of neuromeres as criteria of metamerism will therefore largely depend upon the demonstration of the metameric value of the hindbrain segments, concerning whose number there is very little disagreement. For in this region there is no danger of confusing the anlagen of adult organs with the ancestral nervous segments. What, then, is the argument in favor of the morphological value of neuromeres?

The general argument in favor of the metameric importance of neuromeres may be stated briefly as follows:

1. The ancestors of Chordates were metameric animals with a metameric nervous system such as is seen in Annelids.

2. In accordance with the fundamental law of biogenesis, this ancestral metamerism or neuromerism would be expected to manifest itself ontogenetically, and possibly transiently, in the central nervous system of vertebrate embryos.

3. Such a segmentation affecting all regions of the nervous system has been described by investigators, notably Locy ('95) and Hill ('00), as occurring in the embryos of many classes of Chordates.

4. In the region of the medulla the neuromeres or rhombomeres appear to have constant relationships, through the intermediation of nerves, with the visceral arches. The metamerism of the latter would therefore seem to indicate a corresponding metamerism of the neuromeres.

None of these assertions, however, is beyond cavil; none is undisputed. Not all morphologists conclude that the ancestors of Chordates were metameric animals. The possibility of the independent acquisition of metamerism by Chordates is by no means excluded and the application of the biogenetic law in the interpretation of neuromeres loses all significance, except upon the assumption of a metameric ancestry of Chordates. A relatively large number of investigators have questioned the results of Locy and Hill both as to their accuracy and as to their interpretation. The serial homology of the rhombomeres (connected with the cranial nerves) with the neural segments anterior and

posterior to the medulla remains yet to be proved. A rhombomere—connected with visceral-arch musculature by means of a splanchnic motor nerve—may be a structure sui generis. The observations of Gräper ('13) indicate that the nervous connections of the rhombomeres with the visceral arches are not simple metameric relations like those of spinal motor nerves to somatic musculature. Moreover, the structural resemblance of rhombomeres and myelomeres has been disputed (Neal, '98, pp. 176, 185).

Indeed, the hypothesis of the phylogenetic significance and metameric value of the neuromeres meets with a number of serious difficulties. These are:

1. The surprising fact that neuromeres are more conspicuous in the embryos of higher, than they are in the embryos of lower Chordates. Neuromeres are wanting in the embryos of the most primitive of all Chordates—Amphioxus. They are extremely irregular and usually asymmetrical in that very primitive type of Cyclostome—Bdellostoma (Dean). In Petromyzon the rhombomeres are scarcely discernible, even by one thoroughly familiar with them in the embryos of higher Chordates. Myelomeres have never been described in this form. The rhombomeres are less pronounced in embryo Elasmobranchs than in those of reptiles, birds, and mammals. Such evidence accords better with the supposition that the neuromeres are a coenogenetic acquisition by Chordates than with the hypothesis that they are remnants of an invertebrate neuromerism. Furthermore, it is wholly illogical to infer that because neuromeres are ancestral characters in the Amniota, that they are such in the Anamnia.

These facts are the more significant when contrasted with the facts presented by the mesodermic segmentation. As would be expected in the case of an 'ancestral' metamerism, assumedly affecting the entire body of the organism, the mesodermic somites of Amphioxus form an unbroken series of metameric segments relatively more pronounced than in any of the higher Chordates. In Petromyzon embryos, likewise, the mesodermic metamerism is continuous throughout head and trunk and, as

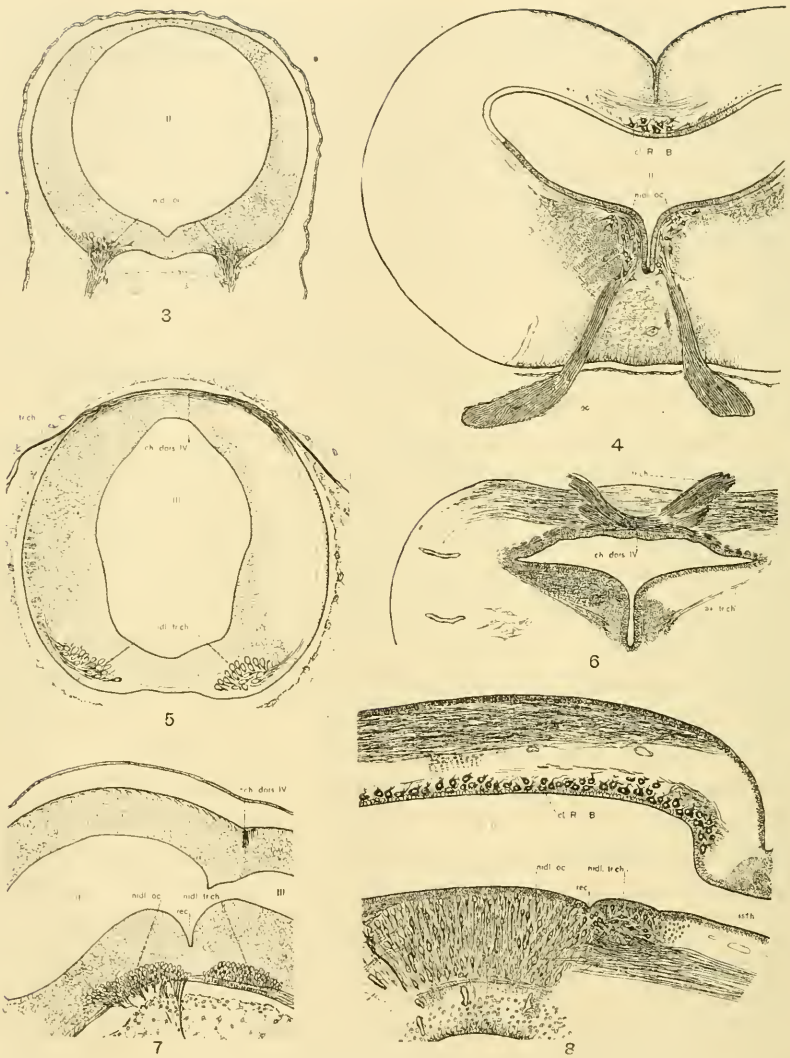


Fig. 3 A portion of a frontal section of a 13-mm. Squalus embryo, showing the nidulus of the oculomotor nerve in the floor of the midbrain vesicle (II). The neuraxon processes of the neuroblast cells may be traced into the root of the nerve (*oc.*).

Fig. 4 A portion of a cross-section of a Squalus embryo ('pup' stage) in the region of the optic lobes, showing the relations of the nidulus (*nidl.oc.*) of the oculomotor in the floor of the brain. A compact bundle of axons may be traced from the nidulus into one of the roots (*oc.*) of the nerve. The ganglion cells of the oculomotor lie near the median ventral sulcus of the midbrain and appear to be actually nearer the ependyma than in the earlier stage shown in figure 3.

The comparison of figures 3 and 4 suggests that there has been an ontogenetic migration ('neurobiotaxis' Uriens-Kappers) of the oculomotor nidulus toward the lumen of the midbrain. Multipolar ganglion cells (*cl.R.B.*) have appeared in the dorsal wall of the midbrain near the median line and the ependymal lining. The neuraxon processes of these cells enter the mandibular branch of the trigeminal nerve(see figs. 1 and 2).

Fig. 5 A frontal section of an 18 to 19-mm. Squalus embryo in the region of the isthmus, showing the trochlearis nidulus and dorsal chiasma. At this stage most of the trochlearis nidulus lies within the first (cerebellar) rhombomere, but also extends anteriad into the region of the midbrain. Since, however, the larger number of the neuroblasts of the trochlearis are situated in rhombomere 1, the nerve is clearly a hindbrain nerve and must be assigned to that part of the brain in schemes of metamerism.

Fig. 6 A cross-section of a Squalus embryo ('pup' stage) in the region of the isthmus and of the trochlearis chiasma (*ch.dors.IV*). As a result of the displacement anteriorly, the trochlearis nidulus does not appear in cross-sections of the brain at this stage. The section, however, shows some of the trochlearis neuraxons (*ax.tr'ch.*) in the course of their ascent from a fiber tract lying near the median ventral sulcus of the floor of the midbrain. These may be traced in subsequent sections to their union with the dorsal chiasma and in the floor of the midbrain to the central nidulus which, through a forward shifting of the ventral wall of the brain, now appears as the floor of the midbrain region.

Fig. 7 A parasagittal section of an 18-mm. Squalus embryo, showing the relative positions of the oculomotorius and trochlearis niduli. The position of the trochlearis chiasma in the dorsal wall of the brain (*ch.dors.IV.*) marks the division between midbrain and hindbrain vesicles. The lateral recess (*rec.*) in the floor of the brain lies within the limits of the midbrain vesicle and does not indicate a boundary between the primary brain divisions. This fold in the lateral wall makes the niduli of the oculomotor and trochlearis nerves appear in the section to be more distinct than they actually are by bending the somatic motor column laterally out of the plane of the section. Most of the trochlearis nidulus lies well within the bounds of the hindbrain (first rhombomere). The elongated nidulus of the oculomotorius is only partially shown in the section.

Fig. 8 A parasagittal section of a Squalus embryo in the region of the midbrain ('pup' stage). At this stage, through the displacement of the floor of the brain, the trochlearis nidulus appears to lie well within the limits of the midbrain, in close proximity to the nidulus of the oculomotorious. Their relations to the lateral recess resemble those seen in the earlier stage (fig. 7). The boundaries of the two niduli, however, are not very distinct, and it is possible to trace neuraxon processes of neuroblasts anterior to the recess posteriorly and dorsally toward the trochlear chiasma. In other words, the niduli of the two nerves overlap each other, schemes of metamerism notwithstanding. The Rohon-Beard cells are conspicuous in the dorsal wall of the brain. All figures are based on camera drawings of embryos of Squalus acanthias prepared by the Ranson-Cajal method. Abbreviations: Figures 3 to 8. *II,III*, Second and third neuromeres (Neal, '96, '98); *ax.tr'ch.*, axons of the trochlearis; *cl.R.B.*, Rohon-Beard cells of midbrain; *isth.*, isthmus; *midl.oc.*, nidulus of the oculomotorius; *nidl. tr'ch*, nidulus of the trochlearis; *oc.*, oculomotorius nerve; *rec.*, recess in floor of midbrain; *tr'ch.*, trochlearis nerve.

in Amphioxus, all of the somites give rise to permanent myotomes (Koltzoff, '02; Neal, '17). An homologous metamerism of the mesoderm is found in Elasmobranch embryos (VanWijhe, '82, and many others), but some of the somites in the head region are greatly reduced and two or three fail to differentiate permanent myotomes. Cephalic somites are even less distinct in Amphibian embryo (Platt, '94, '97). In Amniote embryos the only remnants of the pre-otic mesodermic segmentation are seen in the 'head-cavities' which give rise to the eye muscles. Such evidence accords perfectly with the assumption that the mesodermic metamerism was characteristic of the ancestors of Chordates. The contrast with the neuromeric segmentation makes it difficult to believe that it represents a similar ancestral metamerism. That morphologists will consider this difficulty removed by the denial that the Leptocardii and Cyclostomes are primitive types of Chordates is doubtful, for the majority of morphologists believe that Amphioxus is the contemporaneous form which most closely approximates the appearance of the ancestors of Vertebrates. The absence of neuromeres in this form, therefore, constitutes a serious objection to the hypothesis that neuromeres are "the last remaining remnants of an ancestral metamarism."

2. The striking structural and morphological differences between the so-called neuromeres in the different regions of the central nervous system are not in accord with the supposition that they represent a series of homologous segments. On the basis of their structure, there is not the slightest evidence that the neuromeres of the spinal cord ('myelomeres' of McClure, '90) are other than the passive result of the mechanical pressure of the adjacent mesodermic somites. They are limited to the ventrolateral wall of the neural tube and they appear and disappear in correlation with the appearance and disappearance of the rounded contour of the adjacent somites. They are least pronounced in the region of the neck where the somites are least developed. The contrast with the 'rhombomeres' is striking and significant. No simple mechanical interpretation of the latter is possible. For the rhombomeres are not only local ex-

pansions of the entire wall of the hindbrain, but, in Squalus embryos at least, they involve local paired thickenings of the lateral walls of the medulla. The rhombomeres, moreover, do not alternate regularly with the mesodermic somites and they persist long after all traces of adjacent somites have disappeared. Their peculiarities are so distinctive and so strictly limited to the region of the medulla oblongata that they appear to have arisen in adaptation to local conditions. For this reason, both the writer ('98) and Streeter ('08) have ventured the opinion that the rhombomeres have arisen in correlation with the visceral arches with which they are functionally connected by means of visceromotor nerve-fibers. Therefore, since the metamerism of splanchnic musculature is limited to the pharyngeal region of Chordates, the possibility that the neural segments which have arisen in association with the visceral arches have no exact homologues in other regions must be granted.

The only neural structures anterior to the medulla which may be compared with the rhombomeres and considered as criteria of metamerism are the primary forebrain and midbrain segments. For they are the only neural segments which correspond numerically with mesodermic segments and which involve all of the neural tube, ventral and dorsal, as do the rhombomeres. That there were formerly visceral arches corresponding with these two neuromeres has been assumed for reason by most morphologists. The conflicting results of investigators do not justify the opinion that there are more numerous neuromeres in this region. After more than a generation has passed since the neuromeres were asserted to be of morphological importance and considered as trustworthy criteria of the metamerism of the head we find morphological opinion hopelessly divided regarding the nature and number of neuromeres in the forebrain and midbrain region. Were we to accept the assertion of Johnston ('05) that the results of Locy ('95) and Hill ('00) "may be taken to represent the present state of knowledge of the neuromeres," we should be obliged to ignore the divergent conclusions of Eycleshymer ('95), Neal ('96, '98, '14), Kingsley ('97), Kupffer ('06), Wilson and Hill ('07), Griggs ('10), Gräper ('13), Smith ('14).

As a matter of fact, the proof that a serially homologous segmentation extends throughout the entire length of the nervous system has not yet been given. The evidence on the whole seems to the writer to favor the view that a rhombomere is a structure sui generis, although in their numerical correspondence with the mesodermic somites there is evidence of the metamerism of the rhombomeres. Their nerve relations, however, weaken the force of this evidence.

3. It is difficult to reconcile the assumption of the primitive metamerism of the rhombomeres with the facts concerning their nerve relations given by Gräper ('13) and presented in this paper. These are summarized in figures 1 and 2. If there ever were in the ancestors of Vertebrates simple, metameric, 'one-to-one' relations of nerve and muscle, such connections have been profoundly modified in the course of phylogenesis. The changes have influenced the visceromotor system of fibers not less than the somatic-motor system described in a former paper (Neal, '14). Figures 1 and 2 show that visceromotor fibers from four rhombomeres (4 to 7) innervate the musculature of a single visceral arch (the second, or hyoid). A single rhombomere, such as the sixth or seventh, may be the source of the motor fibers of two visceral arches. A single visceral arch, such as the first or third, may receive motor fibers from two rhombomeres. The relationships are equally non-metameric and puzzling in the case of the somatic-motor fibers. Two successive myotomes—Van Wijhe's second and third—are innervated by fibers which arise from, or have their niduli in, rhombomeres which are separated from each other by three or four intermediate rhombomeres. The abducens, in other words, in reaching the external rectus muscle crosses two mesodermic somites and two cranial nerves, the facialis and the trigeminal. These are certainly not the expected relationships of correlated metameric elements. Upon the assumption of a primary and unchangeable connection between nerve and muscle, it would seem impossible in the light of such evidence to regard the rhombomeres as metameric structures In the light of what we know, however, regarding the free outgrowth of nerve-fibers, the motor nerve relations may possibly be interpreted as the result of a process of nerve substitution.

The peculiar relationships of the abducens nerve have been discussed in an earlier paper (Neal, '14, pp. 118–122), and have been found reconcilable with the hypothesis of the primary metamerism of the neuromeres (rhombomeres). The fact that Gräper ('13) finds the nidular relations of the cranial nerves of mammals identical with those described in this paper for Elasmobranchs would seem to exclude the likelihood of a phylogenetic migration of the motor niduli. The ontogenetic shifting of the relative position of the visceromotor and somatic-motor niduli occurs in Squalus precisely as in mammals (Gräper, '13). This is shown in figures 9 to 13 of this paper. But this migration involves no metameric shifting of the motor niduli from one neuromere to another. The nerve relations described above, moreover, may not be explained as the result of a metameric shifting of muscles associated with the cranial nerves, since of such migration there is neither comparative anatomical nor comparative embryological evidence. Consequently, upon the assumption of an original metamerism of the rhombomeres and of their associated muscles, it is necessary to assume that the motor cranial nerves have invaded territory once foreign to them.

It is not easy, however, to discover the conditions which would lead to such a series of nerve substitutions. Comparative embryology and anatomy throw little light upon the problem. The problem presented by the relations of the abducens has already been discussed at length by the writer ('14). The nidular relations of the facialis nerve (figs. 1 and 2) are equally hard to interpret. Assuming the original metamerism of the rhombomeres, why should motor fibers of four (4 to 7) rhombomeres be distributed to a single visceral arch? In Amphioxus, the fibers of four metameric nerves are distributed to the velar musculature. How this plexus arose is uncertain. Its appearance may be correlated with the enormous backward extension and later recession of the mouth in larval Amphioxus. If a similar backward extension of the mouth occurred in the ancestors of Vertebrates and a similar plexus of four metameric nerves were formed, the results would resemble in all essentials the relations shown by the facialis. Ontogenetic support for such a supposition, however, is wholly lacking.

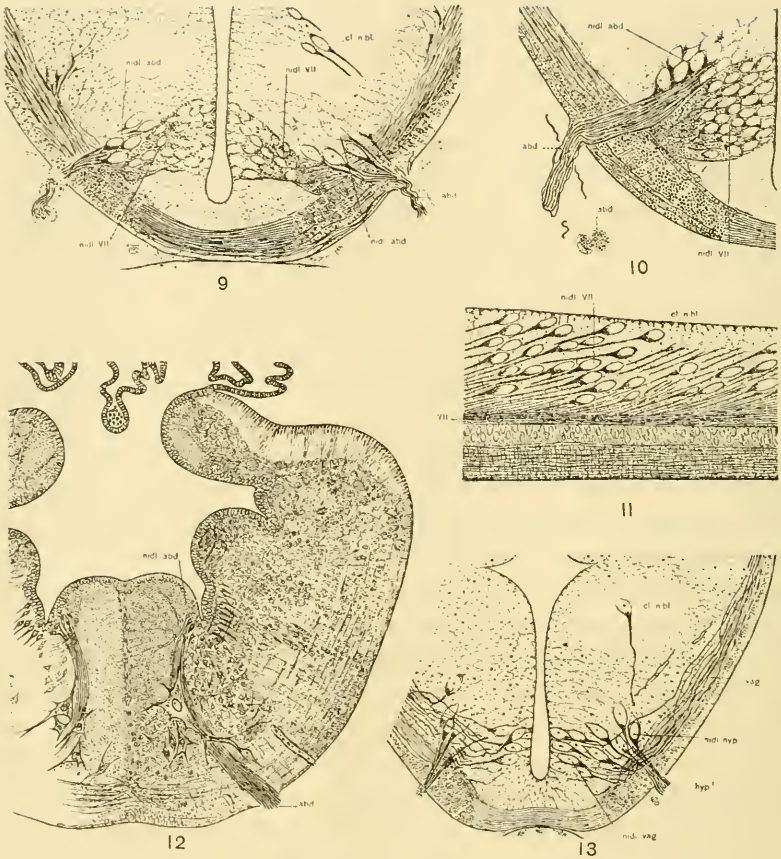


Fig. 9 A portion of a cross-section of a 13-mm. Squalus embryo cut in the region of the sixth rhombomere, showing the primary relations of somatic motor and splanchnic motor niduli. These are just the reverse of those described in text-books. Subsequent migration (neurobiotaxis) brings them into the adult position in relation to each other.

Fig. 10 A section of the same embryo in the region of one of the anterior roots of the abducens, showing the niduli of the facialis and of the abducens in their primary position. A compact bundle of neuraxons of the abducens passes dorsal to the nidulus of the facialis. The left wall only is shown.

Fig. 11 A portion of the left wall of the medulla as seen in a frontal section of a 13-mm. Squalus embryo, showing a portion of the nidulus of the facialis (r.hyoideus). The neuraxon processes of the numerous neuroblasts extend anteriorly and laterally in the brain wall. They unite into a longitudinal fiber tract which extends anteriorly to the root of the facialis nerve. This fiber tract is quite independent of the marginal veil of fibers.

The relation of the first visceral (mandibular) arch to two rhombomeres (rhombomeres 2 and 3) might seem to support the opinion—for which further evidence has been adduced—that the mandibular arch is morphologically double, a result which would follow from the disappearance of an intermediate gill-cleft. The force of this evidence, however, is weakened by the fact that the third visceral arch—which no morphologist regards as double—is also connected by nerve-fibers with two rhombomeres (rhombomeres 6 and 7). The nerve relations of the rhombomeres, therefore, appear to throw very little light upon the complex problem of the metamerism of the vertebrate head. The motor nerves, however definitely related to particular visceral arches, show no similar limitation to individual rhombomeres. There seems to be no correlation between nidular boundaries and rhombomeric boundaries. So far as it goes, this evidence militates against the hypothesis of the metameric significance of neuromeres.

In the historical discussion of the neuromeric problem the constancy of the relationship of particular nerves to individual neuromeres has always been considered a point in favor of the hypothesis of the metameric significance of rhombomeres. It is a fact that the anlagen of individual cranial nerve ganglia are proliferated from individual rhombomeres in all classes of vertebrates, as stated by the writer ('96, '98). The relations of the

Fig. 12 A portion of a cross-section of the medulla in the region of one of the roots of the abducens, showing the position of the nidulus (*nidl.abd.*) in the 'pup' stage. Like the oculomotorius nidulus, that of the abducens seems to have migrated centrad ontogenetically. Large multipolar ganglion cells lie in the wall of the medulla in close proximity to the neuraxons of the abducens, but evidence that such cells form a part of the abducens nidulus is wanting.

Fig. 13 A portion of a cross-section of a 13-mm. Squalus embryo in the region of the first hypoglossus root, showing the relations of splanchnic motor and somatic motor niduli. They are seen to be the same as those in the region of the abducens-facialis complex. Here, as there, the two sorts of neuraxons cross each other within the wall of the neural tube. All figures are based on camera drawings of embryos of Squalus acanthias preserved by the Cajal-Ranson method. Abbreviations: *VII.*, motor fiber tract of the facialis; *abd.*, abducens nerve; *cl.nb'l.*, neuroblast; *hyp.*[1], anterior root of the hypoglossus; *nidl.abd.*, abducens nidulus; *nidl.VII.*, facialis nidulus; *nidl.hyp.*, nidulus of the hypoglossus; *nidl. vag.*, nidulus of the vagus; *vag.*, vagus neuraxons.

roots of these nerves to the rhombomeres are also constant. The central niduli of origin of these nerves are found to be the same in mammals (Gräper) and in the Elasmobranchs. Of the constancy there seems to be no doubt whatever. But the constancy has significance chiefly as evidence of the constancy of individual rhombomeres and not as proof of their metameric value. The significant fact is that the constant nidular relationships of the cranial motor nerves are not such as to justify the prevalent assumption of their metamerism. They certainly do not correspond with the relationships assumed in many schemes of cephalic metamerism, including those of the writer ('98 a, '98 b) and Johnston ('05).

In the light of the foregoing evidence, are we to accept neuromeres as trustworthy criteria of the metamerism of the vertebrate head? If we do, we must not only shut out eyes to the difficulties and objections just mentioned, but we shall also need to answer the question, Whose neuromeres shall we accept as criteria of metamerism? Shall they be those of Kupffer ('85) or those of Locy ('95) and his pupil, Hill ('00), those of the writer ('96, '98), or those of Griggs ('10)? Even if we disregard the evidence of the non-metameric nerve relations described in this paper and the lack of numerical correspondence of neuromeres and branchiomeres, there remains the unanswered question, Which neuromeres are the 'true' neuromeres? In the opinion of the writer, this question may not be answered regardless of the metamerism of the mesoderm, as has been so often done by students of neuromerism. The metamerism of the mesoderm is primary while that of the nervous system is secondary. There must therefore have been at one time a correspondence between the neuromeric and mesomeric segmentation of the head. Such a correspondence obtains for the primary brain divisions of Squalus (neuromeres I to VII of the writer) and the mesodermic somites. In this numerical correspondence may be seen the most convincing evidence of the metameric value of neuromeres.

Of the metamerism of the mesoderm there is no resonable doubt in the light of the very general agreement among morphologists concerning the mesodermic somites of the head. The

mesomerism described by VanWijhe ('82) in his famous monograph has been repeatedly confirmed for Elasmobranchs by Hoffman ('94), Neal ('96, '98), Sewertzoff ('98), Braus ('99), and Johnston ('09). An homologous mesomerism in Cyclostomes has been described by Koltzoff ('02) and Neal ('18) and in Amphibian embryos by Miss Platt ('97). Such a consensus of opinion contrasts strikingly with the divergence of opinion regarding neuromerism, and the mesodermic somites must therefore be considered as the most trustworthy criteria of metamerism. The significance of the numerical correspondence between the 'primary neuromeres' of the brain and the cephalic somites is therefore obvious.

Several writers who have discussed the neuromeric problem have expressed surprise that the writer ('96, '98) has considered the three anterior brain vesicles (neuromeres I to III, figs. 15 to 17) as true neuromeres and as serially homologous with the rhombomeres. The reasons for this divergent opinion of the writer have already been given, but they may be summarized here. In the first place, there is the significant fact that in the embryos of remotely related Vertebrates such as cyclostomes, Elasmobranchs, and birds the brain consists of seven primary segments (figs. 15, 16 and 17). In Squalus embryos there is a numerical correspondence between these neural segments ('neuromeres') and mesodermic somites anterior to the sixth, counting the anterior cavity of Miss Platt as a mesodermic segment. In this numerical correspondence with metameric structures, such as the somites, we have a second significant fact favoring the conclusion that the primary neural segments are the true neuromeres. In the third place, the primary anterior brain vesicles ('neuromeres' I and II) are structurally comparable with the rhombomeres, while their secondary subdivisions are not—at least some are not. The criticism of this conclusion of the writer on the ground that the three anterior vesicles are considerably larger than the four (or five) posterior ones (rhombomeres) does not seem to have much weight, since the increase in size of the anterior 'neuromeres' on account of their increased functional importance would naturally be expected. Such, in

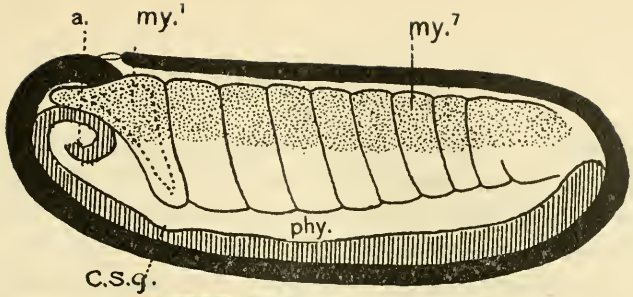
Figs. 14 to 17 represent a series of Chordate embryos showing the increasing conspicuousness of the 'neuromeres' and the associated reduction of somites in the head region. The evidence favors the conclusion that neuromeres are a coenogenetic acquisition of the Chordates. The mesodermic segmentation, however, bears the earmarks of an ancestral segmentation which degenerates during the phylogenesis of the Chordata.

Fig 14 A larval Amphioxus after Hatschek ('81), showing the unbroken series of mesodermic cavities, from each of which is differentiated a permanent myotome. 'Neuromeres' are wanting. If 'neuromeres' were an ancestral characteristic like the mesomeres, how is the absence of the former and the presence of the latter to be explained? None of those who have assumed the metameric importance of the neuromeres have ever given an answer to this important question.

Fig. 15 A diagram of larval Petromyzon, showing the continuous series of myotomic divisions, each of which, as in Amphioxus, forms a permanent myotome and persists in the adult (Koltzoff, '02). In the diagram the somites are represented as they appear in an eight-day (Naples) embryo, while the neuromeres appear (greatly exaggerated in the diagram) as in a twelve-day embryo. In later stages neuromere III secondarily subdivides into two segments—'rhombomeres' 1 and 2 auctorum. The reason why the primary segment and not its secondary subdivisions is considered to be a true neuromere is given in the context.

Fig. 16 A parasagittal section of a 4-mm. Squalus embryo, showing the seven anterior primary brain segments (considered by the writer as the true Vertebrate 'neuromeres') and the series of mesodermic somites—the exact homologues of the series shown in Amphioxus and Petromyzon. As in the latter animal, neuromere III subdivides secondarily to form the 'rhombomeres' 1 and 2 (see figs. 1 and 2). From all of the somites, except somites 4 and 5, myotomes are differentiated. The myotome of somite 6 is transient, however, and that of somite 7 forms the first permanent postotic myotome. The myotomes of somites 1 to 3 form the eye-muscles (Neal, '18). Yet, while the degeneration of cephalic myotomes has thus begun in Elasmobranchs, the 'neuromeres' are much more conspicuous than in Cyclostomes.

Fig. 17 A parasagittal section of a chick embryo of 13 or 14 somites, showing the conspicuous neuromeres (I to VII), homologous with those of Petromyzon and Squalus, and the disappearance of true somites anterior to the seventh (*my.*^{7}). A noteworthy fact is the later secondary subdivision of 'neuromere' III into two segments, the exact homologues of 'rhombomeres' 1 and 2 of Petromyzon and Squalus. Somites 1, 2, and 3 of Anamniote embryos are represented in birds by the three 'head-cavities' which form the eye muscles. Abbreviations: *I–VII.*, neuromeres 1 to 7; *a.*, anterior entodermic diverticulum (the homologue of the 'anterior' head-cavity of Elasmobranchii and Ganoidei; *c.s.g.*, anlage of the 'club-shaped gland' (first pair of visceral pouches in Amphioxus); *my.*^{1}, *my.*^{2}, *my.*^{3}, . . . *my.*^{7}, myotomes 1 to 7; *phy.*, pharyngeal region; *sp.*, spiracle.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my.1
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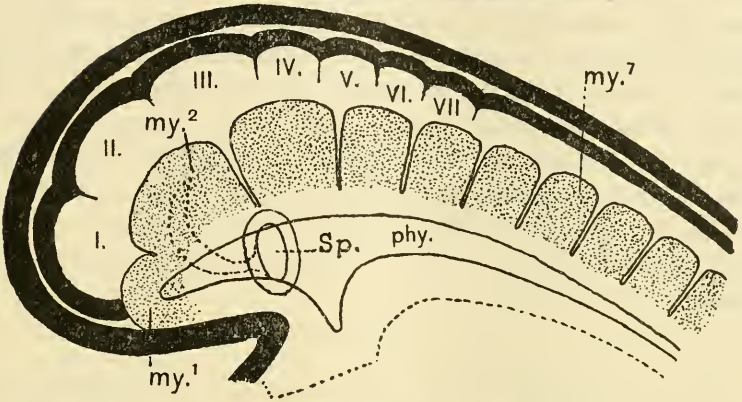
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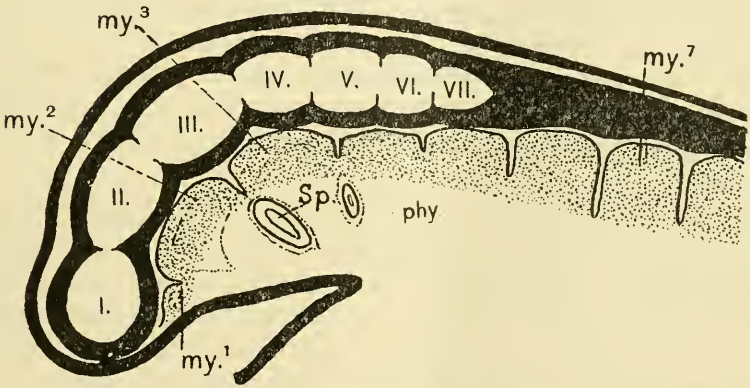
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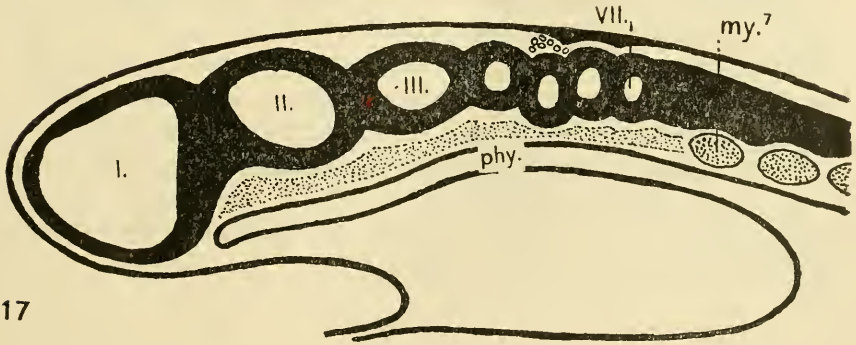
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brief, are the reasons why the writer regards the primary brain divisions as true neuromeres and not their secondary subdivisions. Neuromere III, and not its secondary subdivisions, 'rhombomeres' 1 and 2, figures 1 and 2, therefore, is considered a true neuromere. This conclusion is further supported by the fact that 'neuromeres' II and III contain the motor niduli of the nerves (oculomotor and trochlear) which innervate two successive myotomes (myotomes 1 and 2 of VanWijhe). Such functional relationships of 'neuromeres' and mesomeres are certainly not accidental. But whether the metameric relations of these two 'neuromeres' and myotomes are sufficient to establish the metamerism of the more posterior 'neuromeres' or rhombomeres is doubtful in view of the motor nerve relations of the latter.

SUMMARY

However doubtful the interpretation of the so-called neuromeres of vertebrate embryos in other regions of the body, the hindbrain neuromeres or 'rhombomeres' may be explained neither as anlagen of adult organs nor as the passive results of mechanical pressure of bending of the neural tube. A phylogenetic interpretation of them therefore appears to be not impossible.

None of the assumptions which underlie the phylogenetic interpretation of rhombomeres, however, is undisputed. Not all morphologists assume that the ancestors of Chordates were metameric organisms. Moreover, neuromerism is not seen in the central nervous system of Amphioxus. This fact suggests that neuromerism is independently acquired by Chordates.

In striking contrast with the mesomeric segmentation, the neural segmentation is more conspicuous in the embryos of higher Chordates than in the lower, more conspicuous in the head than in the trunk. Analogous evidence, it will be recalled, led to the abandonment of the vertebral theory of the skull.

The rhombomeres may have arisen, as suggested by the writer ('98), in adaptation to the branchiomeric segmentation. Doubt still attaches to the serial homology of the rhombomeres with other neural divisions (Neal, '98, p. 177).

The results of Locy ('95) and Hill ('00) form a very insecure foundation for a belief in the metameric value of neuromeres. For they are in disagreement with those of Eycleshymer ('95), Neal ('96, '98, '14), Kingsley ('97), von Kupffer ('06), Wilson and Hill ('07), Griggs ('10), Gräper ('13), Smith ('14).

The neuromuscular relations of the rhombomeres described in this paper and in a former paper ('14) are hard to reconcile with the assumption of the metameric value of neuromeres. Even with a strong prejudice in favor of the assumption, the central nidular relations of the rhombomeres are such as to throw much doubt upon the belief that neuromeres are trustworthy criteria of the metamerism of the head.

No such doubt attaches to the segmentation of the mesoderm which manifests in the embryos of lower Chordates a series of homologous segments concerning which observers are in very general agreement. The mesodermic somites therefore afford the most reliable criteria of the primitive metamerism of the head. It is a wholly gratuitous assumption to assert that myotomes have disappeared in phylogeny anterior to the first permanent myotome of lower Chordates.

Consequently, until some evidence to the contrary is presented, the writer feels compelled to retain the opinion expressed many years ago, that the chief evidence of the metameric value of neuromeres consists in their numerical correspondence with the mesodermic somites. Such a correspondence, however, obtains in the head region of vertebrates for only the primary brain vesicles (neuromeres I to VII of the author) and not for the secondary subdivisions of these (such as rhombomeres 1 and 2, which result from the secondary subdivisions of neuromere III). The larger size of the three anterior vesicles (I to III) presents no difficulty to this view, since this difference may be attributed to the increased functional importance of these brain divisions.

Except in the case of 'neuromeres' II and III (Neal), however, the motor nerve relations of the neuromeres do not accord with the supposition that these are metameric structures.

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THE GILL-CHAMBER OF DRAGONFLY NYMPHS[1]

STEPHEN G. RICH

Entomological Laboratory of Cornell University

FORTY-EIGHT FIGURES

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INTRODUCTION

Darwin calls attention to the remarkable differentiation of the alimentary tract in the nymphs of dragonflies, mentioning the adaptation of the foregut for chewing and the hindgut for respiration and propulsion. Probably most entomologists have seen the elongate nymphs of some Aeschnid dragonfly spout water from the rectum in a jet at the surface or have seen the creature dart through the water under the propulsion of this same jet. A nymph, placed in a watch-glass of water, in a few minutes, when it has become quiet, will inhale and exhale water through the anal opening; a little suspended matter makes the currents quite clear. That the rectum is in these forms much enlarged and that it is furnished with tracheal gills is well known; the respiratory structures themselves have been

[1] Contributions from the Entomological Laboratory of Cornell University.

studied in a few isolated forms. The present study is intended to cover this field comparatively. Twenty-one genera, comprising all save the most rare ones of the Cayuga fauna, have been studied. The work was undertaken at the suggestion of Dr. J. G. Needham, to whom I am much indebted for material and for critical advice. Through him I was able to see the unpublished work of Miss Andrews, to which I refer often. Dr. Philip P. Calvert, of Philadelphia, allowed me the use of some sections of Calopteryx and a stained and mounted rectum of Cora chirripa. Dr. W. A. Riley and Dr. Needham loaned me sections of Libellula, and Mr. E. A. Richmond, of Cornell University, provided some excellent Corduline material from New England.

MATERIAL AND METHODS

The greater part of the work here described was done by dissection under the binocular microscope. The higher powers of the prismatic binocular were ample for all work save the details of tracheation. The dissections were supplemented by sections in a few cases, notably in Calopteryx and Libellula.

Fresh material was, in the main, the most satisfactory, but that which had been preserved in 2 per cent formalin was in most cases almost as good. In it fat stood out better and tracheation was not lost. Alcoholic material was usually in bad shape, being badly contracted and having only the larger branches of the tracheae visible.

HISTORICAL REVIEW

According to Hagen ('52) the nymphs of Anisoptera were possibly first observed by Aristotle, under the name of 'Orsodacna.' Charlton (1671), Rondelet (1555), and Swammerdam (1686) were the first among the moderns to observe and describe the nymphs. Rondelet calls them 'water cicadas.' The first recognition of their larval character was by Swammerdam. Poupart (1702) was, without doubt, the first to see the anisopteran nymphs take water into their rectum, and Reaumur (1742), repeating the observation of Poupart, also noticed the enlarged branchial rectum, as did Roesel in 1744.

Cuvier (1799) was the first to investigate the structure of the gill-chamber. In an 'Aeshna' (which probably was Anax imperator), he found twelve rows of gills bearing villi in the much enlarged rectum and observed fine tracheal branches in the villi.

Haussmann (1803), Sprengel (1815), Marcel de Sorres (1818), and Meckel ('29) add nothing but curious errors. Suckow ('28) describes with more accuracy than any one preceding him the rectum of another species of Anax.

The more adequate study of the anisopteran rectum begins with the work of Leon Dufour ('49, '52). In the first paper Dufour claims that the zygopteran nymphs also have rectal gills, but later admits that there are such only in Calopteryx. He treats in detail two aeschnine species and one libellulid. Hagen ('52), summarizing the work up to that date, corrects the crude taxonomy of Dufour, for whom, as for most of his precursors, every big aeschnine nymph seems to have been 'Aeschna grandis.'

The work of Oustalet ('69) set a new standard of precision in observation. He described with care the distribution of tracheae from the main trunks to the rectum and figures this well. In Anax and Libellula he adds a number of details of gill structure, being the first to describe the loops of tracheoles in the gills. He notes also the existence of a distinct anal canal in the Aeschninae only.

Since the work of Oustalet, some nineteen papers have been written on this subject. The most important are those of Chun (,77), in which epithelial cushions are first noted; Poletjev ('80) described several new forms of gills; Sadones ('95) gives a fine histological study of the whole alimentary tract of Libellula depressa. Scott ('05) described the tracheation of the nymph of Plathemis lydia; Ris ('13) described gill forms of ten genera and some features of their rectal anatomy, but without figures; and Oguma ('13) described the disappearance of the gills during metamorphosis.

Specimens of the following genera have been examined by the workers above quoted: Onychogomphus, Gomphus, Aeschna (S. lat), Brachytron, Anax, Cordulegaster, Epitheca, Cordulia, Sympetrum, Libellula, Plathemis.

Throughout the literature of the subject there is a tendency grossly to overestimate the number of gills in each rectum. The statement of Oustalet that one species of Anax has 50,000 villi is copied into the standard text-books: Scott ('05) seems to think that Plathemis has as large a number of gills. The table, included in my summary, will show the facts as I have found them.

THE GILL-CHAMBER OF CORDULEGASTER

The gill-chamber of Cordulegaster diastatops serves as a rather primitive form, by reference to which the features of other genera may be described with ease and clearness. The present description is from a dissection of a full-grown nymph, freshly killed.

A very large tracheal trunk lies on each side of the rectum and extends to a point near its caudal end. Here this trachea passes ventrad and unites with a similar but smaller ventral trachea. I call these two, dorsal and ventral trunks, following the accepted usage. Near where the dorsal bends around into the ventral trunk, a thinner trunk is given off, which runs along the lateral margin of the abdomen. I call this the 'lateral trunk.'

As shown in figures 1 and 2, large tracheae continue the dorsal and the ventral trunks caudad. These are respectively the 'postdorsal' and 'postventral tracheae.' The postdorsal on each side sends one of its divisions to the extreme caudal part of the rectum, the anal canal; the postventral does not supply parts so far caudad. The lateral trunk does not share in any way in the tracheation of the rectum.

These tracheal trunks, at least at their thickest parts, are enveloped by a whitish patch of adherent fatty tissue. In some genera (Ophiogomphus in particular) this is fused into the general fat-body occupying the haemocoele, and I am inclined to think that it is always a part of this, though detached from the rest.

The rectum itself is a much enlarged portion of the gut, about 2 x 6 mm. in size. It is rounded at the cephalic end, to which the intestine proper, coming from the ventral part of the

haemocoele, is attached. At the caudal end it tapers into a distinct anal canal, about 1 mm. long. On the dorsal side of the rectum are three longitudinal thickened areas, each with about seventeen to nineteen pectinations on each side, and on the ventral side there are three more. One of these areas occupies the middle of the dorsal side. Each area is the base of a set of respiratory structures within the rectum, and is white and opaque. The pectinations point about 45° laterocephalad on the middle dorsal row. Each pectination I call a 'gill base,' and each bipectinate longitudinal area, formed by the fusion of adjacent gill bases, I call a 'double row.' A dense brush of tracheal branches which spring from the two dorsal trunks passes into these gill bases on the dorsal side, but only to the bases. The dorsal trunk is rather suddenly increased in diameter just anterior to the point where the branches to the rectum leave it (fig. 1), but there is no similar expansion of the ventral trunk. No branches to any other organs or parts leave the trunks in the region in which the branches to the rectum are found.

On each dorsal trunk there are eight large branches to the rectum, in addition to the postdorsal trachea. Four of these branches are on the dorsal and four on the ventral side of the trunk. In figure 1 and other figures the branches ventral to the dorsal trunk have been omitted for the most part, as they simply duplicate those on the dorsal side. Where they differ, I note the fact.

Each of the four large primary branches at once splits up: the first one into four or five branches, the second into four, the next two into two or three each. Each of these smaller branches either divides into two or proceeds at once to a gill base, before entering which it again divides dichotomously. One of these branches passes to the anterior or proximal, the other to the posterior or distal end of the gill base of pectination of the double row. From the postdorsal trachea five branches to gill bases are given off on each side. The branches from the dorsal side of the dorsal trunk and the postdorsal trachea pass to the gill bases of the same side of the middorsal double row, and

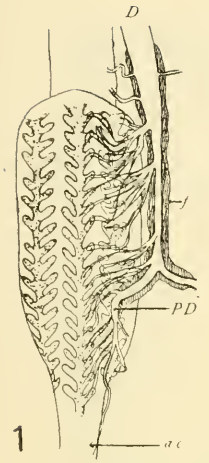
EXPLANATION OF THE FIGURES

Note.—I have omitted in the figures of the rectum viewed from the dorsal aspect most or all of the tracheal branches coming from the ventral side of the tracheal trunks. This has been done to gain clearness and has been merely an avoidance of parts duplicating those figured.

The following are the abbreviations used:

ab, button or pad on cephalic side of gill
ac, anal canal
bf, buttress fold
cb, button or pad on caudal side of gill
cm, circular muscle
cs, epithelial cushion
CT, tracheal connection between dorsal and ventral trunks
D, dorsal tracheal trunk
f, fat tissue
fb, boundary of fat tissue
ff, fleshy fold
g, gill
ia, inferior appendage
L, lateral tracheal trunk
l, lump at tip of gill
la, lateral appendage
lf, longitudinal fold
lm, longitudinal muscle band
mt, Malpighian tubules
PD, postdorsal trachea
pg, pigment
PV, postventral trachea
r, rectum
rb, ridge and bulb on gill
s, setules
sa, superior appendage
si, small intestine
tr, trachea
V, ventral tracheal trunk
v, lobe of anal valve
vi, villus
+, cephalic edge of gill or cephalic end of rectum

Fig. 1 Dorsal view of rectum of Cordulegaster diastatops.
Fig. 2 Ventral view of same.
Fig. 3 Schematic cross-section of same (from Andrews MS.).
Fig. 4 Plan of arrangement of internal folds of same.
Fig. 5 Longitudinal and buttress folds of same.
Fig. 6 Detail of edge of fold of same.
Fig. 7 Musculature and gill bases of Hagenius brevistylis.
Fig. 8 Dorsal view of rectum of Aeschna constricta.
Fig. 9 Longitudinal and buttress folds of same.
Fig. 10 Longitudinal and buttress folds of Basiaeschna janata.
Fig. 11 Longitudinal and buttress fold of Boveria vinosa.
Fig. 12 Double row of Anax junius.
Fig. 13 Gill of same, showing tracheation.
Fig. 14 Gill of same, showing cushions, fat, and pigment.
Fig. 15 Contour plan of double row of same.



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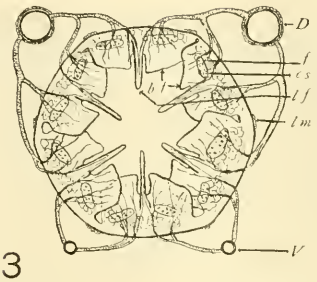

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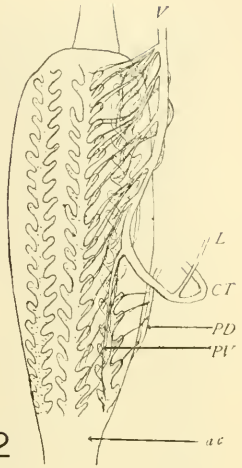

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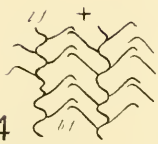

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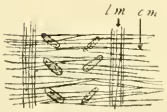

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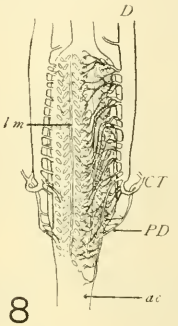

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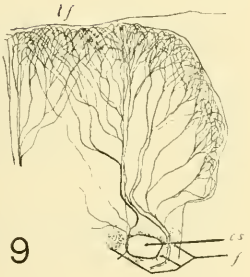

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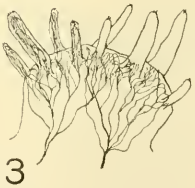

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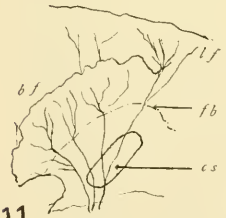

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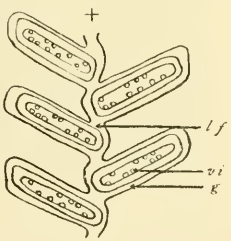

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also to the adjacent gill bases forming the mesal half of the next double row. The lower or more ventral half of this double row and the upper nearer half of the adjacent row on the ventral side are supplied by the similar branches from the ventral side of the dorsal trunk and the postdorsal trachea. The postdorsal here has six branches in some specimens. The tip of the postdorsal passes on to the anal canal.

The arrangement on the ventral side is different. The ventral trunks and the postventral tracheae supply only the midventral double row and the adjacent half of the next row on each side. There are branches on both sides of the ventral as of the dorsal trunk, but each of these divides only to a single row of gill bases (fig. 3). The ventral trunk gives off on each side five branches, each of which divides at once, dividing again just before entering the gill base, exactly as with the branches already described.

Thus there are two single rows and three double rows supplied from the dorsal tracheae, and one double row and two single rows supplied from those on the ventral side. The cross-section (fig. 3, modified from a MS. figure by Miss Elizabeth Andrews) will perhaps make the relations of tracheal trunks to gill bases clear.

If we turn to the interior of the rectum, we shall find there six longitudinal folds, each corresponding with the middle of a double row of gill bases. Each row is supported on each side by a series of buttress folds, which lie over the gill bases. Figure 4 shows this in plan: the buttress folds pass at about 45° cephalad, and are recurved at their tips, overlapping the similarly recurved tips of the adjacent row of folds. These folds, main and buttress, are the actual respiratory parts of the gill-chamber. The buttress folds are alternate on each side of the main fold.

The base of all these folds is filled with a loose mass of fatty tissue, made up of ordinary trophocytes, through which the tracheae pass up into the respiratory organs proper. On the base of each buttress fold are two plates of thick epithelium, one on each side of it. These are oval in shape (fig. 5) and lie across the base of the fold. These, discovered by Chun ('76),

are called 'epithelial cushions' by Sadones, and the name will fit all genera, although originally devised for Libellula, in which these cushions are very much specialized. Their histology is figured and described by Sadones: they consist of elongate cells, fibrillar in structure, beneath the usual thin cuticle. I have indicated also how far up into the fold the fatty tissue reaches. In the main fold the fatty tissue is entirely in the very base of the fold. The cavity within the fold and the spaces among the the fat-cells communicate with the haemocoele, as Sadones ('95) has shown.

Most important is the tracheation of the folds. Each gill base—the base of each buttress fold—receives two tracheal branches, one at each end. The one at the inner end passes up at the point where the buttress fold joins the main fold. The buttress folds are arranged alternately on each side of the main fold which thus receives alternate tracheae from each side. The tracheae which pass up into this point of junction of folds divide into two parts, one of which ramifies out into the main fold, the other of which does likewise in the buttress fold. The other tracheal branch which reaches the gill base branches out into the periphery of the buttress fold. As all these branches approach the edges of the folds, they continue to divide into fine tracheoles, with which the edges are filled. The edges of the folds are thus the parts functional in respiration.

These fine tracheoles are actually imbedded in the epithelium of the fold, as Sadones has shown for two other genera. Contrary to Mackloskie ('83), they do not end blindly, but anastomose and form actual loops. Each ultimate tracheole bends around and loops back into another branch, either from the same tracheal branch or from another. The fringe of loops is continuous along the edge of each fold, irrespective of whence the loop arises. This is indicated in figure 5 and shown in more detail in figure 6.

The edge of the folds appear serrulate on first view, but turns out under higher magnification to be set with chitinized thickenings (fig. 6). The main fold is of uniform height, and slightly waved or plaited (fig. 4). There is a sudden depression at the

end of each buttress fold at the point where it joins the main fold, and the buttress fold ends in a sharp angle here. In figure 3 the longitudinal folds are shown in section.

I was unable to see the musculature of the rectum of this species, but saw it in Hagenius brevistylis, where there are six compact longitudinal bands of muscle, three on the right and three on the left of the middle line of the rectum. They alternate in position with the double rows of gills. The circular muscles, which are outside the longitudinal, are simply a loose series of rings of muscle fiber around the rectum.

FEATURES COMMON TO ALL FORMS

Before passing to a survey of the Anisoptera by genera, it may be well to summarize the points in which the description of the rectum of Cordulegaster, just given, is a description of all the recta studied. The following features are common to all these forms:

1. The distribution of tracheal trunks is the same in all genera. The only differences are in the places and manner in which the trunks become larger or smaller and in the number and grouping of their branches.

2. In the regions in which the branches to the rectum leave the trunks, no branches to other organs originate. From each postdorsal trachea a branch passes to the body wall of the dorsal side, and from the connection between the tracheal trunks a branch passes to the ventral body wall in the eighth and ninth somites.

3. In all forms there are six double rows of gills or buttress folds within the rectum, and a similar number of rows of gill bases appear on the outside. The single rows which go to make up these double rows are not constant in position. They are paired, either into three right and three left or three dorsal and three ventral double rows, or, in some cases, apparently evenly arranged and separated completely from each other.

4. The dorsal trunks and the postdorsals always supply the dorsal two-thirds of the rows of gills. This will be either three full double rows and halves of two more, as in Cordulegaster,

or four full rows, as in certain forms to be described. The remaining rows on the ventral side are of course supplied from the ventral trunks.

5. Each gill or buttress fold is supplied with at least two tracheal branches. In most forms these two branches come from different sources of the different series of major branches leaving the trunk or resulting from the first divisions of these major branches.

6. An epithelial cushion, of tough and thick epithelium, is present on the cephalic side at the base of every gill. In many Aeschnidae a similar cushion is present on the caudal side of the gill or buttress fold also.

7. In the base of all gills or buttress folds fat is present, lying in the cavity of the fold, which communicates with the haemocoele.

8. The rectum always has six bands of longitudinal muscles, arranged three on the right and three on the left side. The circular muscles always form a layer over the whole rectum.

9. In every case the distal part of the folds, gills, or villi are filled with loops of tracheoles, which usually connect fine twigs from each of the branches entering the gill, but some of which also connect twigs from the same branch. This part of each gill is functional in respiration. There are no loops in the region of fat and epithelial cushions.

10. An anal valve, consisting of three semilunar plates, of which one is situated middorsally, is always present.

11. The rectum occupies the seventh, eighth, and ninth somites of the abdomen, but may extend forward into the sixth. It never extends into the tenth somite, but where this is long there is a distinct anal canal. Where this somite is short, the rectum tapers directly and abruptly to the anal valve.

SURVEY OF THE FORMS OF RECTUM

I. FAMILY AESCHNIDAE

A. Subfamily Cordulegasterinae

In this subfamily there is only one genus, represented in the Cayuga fauna by the single species Cordulegaster diastatops, described in detail in the preceding section (figs. 1 to 6).

B. Subfamily Petalurinae

There is no representative of this subfamily in the Cayuga fauna. I am as yet unable to obtain material for study.

C. Subfamily Aeschninae

Of this subfamily we have four genera whose nymphs are common: Aeschna, Basiaeschna, Boyeria, and Anax. The recta of these four genera which I have studied are not distinguishable in any external feature. It is necessary to open them to find any points of difference. The rectum possesses an arrangement of gill bases, a tracheation, and a general appearance identical in all four. Therefore, I describe the external appearance of the rectum in but one of them—Aeschna.

Figure 8 shows the general appearance of the rectum of Aeschna constricta, viewed from the dorsal side. The tracheal branches which leave the dorsal trunks are not grouped or fused at the base as in Cordulegaster, but are evenly spaced or nearly so. Each of these primary branches, which vary in number from ten to thirteen, passes to two or three gill bases of each of two single rows. The most caudal branch before the postdorsal trachea passes to four or five gill bases. The postdorsal is as in Cordulegaster.

There are about twenty to twenty-four gill bases to each single row. They are arranged, as in Cordulegaster, in three dorsal and three ventral double rows, but they are not fused at the center of the row, although in some specimens they are much closer than indicated in the figures. The chief difference be-

tween the tracheation of the gill bases of this form and of Cordulegaster is that here each base receives branches from different primary branches or their divisions, while in Cordulegaster the pair of branches to each base arises from the divisions of only one primary branch.

The tracheation of the ventral side is similar to that of the rectum of Gomphus descriptus (fig. 16). The gill bases are as in figure 8, and the large number is accommodated by each branch dividing once more than in Gomphus. There is the same even spacing of branches as on the dorsal side. The postventral is much less prominent than in Cordulegaster, and in the Gomphinae reaches its smallest extent.

Within the rectum of Aeschna constricta is the same type of longitudinal and buttress folds as in Cordulegaster, but with slight change of form. They are rounded and lack the nicks separating them from the main fold. They are as tall as the main fold, and in some few cases a trifle taller. Near the ends of the rectum they become less prominent. Figure 9 shows a typical portion of the folds, with cushions at the base on both sides, as in Cordulegaster, fat indicated and tracheation shown. There are six folds running longitudinally, and twenty-four more or less buttress folds on each side of each. The folds are in three dorsal and three ventral double rows, exactly as in Cordulegaster, and homologous therewith. Ris ('13) found folds like these buttress folds in Aeschna cyanea.

In Basiaeschna janata (fig. 10) the buttress folds have taken a new shape. They are narrowed at the base, and the cephalodistal corner is prolonged into a sharp angle. The cushions are here somewhat twisted around and upwards. Otherwise the structure, in every respect, is as in Aeschna.

Boyeria vinosa (fig. 11) has a somewhat different form of buttress folds. The fat comes further up into it. The cushions are twisted as in the last form, but the fold itself is rounded along the top edge and bears distally a slightly recurved and downpointing apex. It is also more wrinkled as it lies in the rectum than are the forms hitherto described. It is distinctly lower at its inner edge than is the longitudinal fold and shows a slight notch at the point of junction with the longitudinal fold.

In all three of these forms the bases of the gills are set at the same angle as in Cordulegaster. They point 45° cephalad from a transverse line. In the next form they lie 10° nearer a transverse position, but this is not noticeable from the outside of the rectum.

Anax junius (figs. 12 to 15) possesses a rectum that is exactly like that of Aeschna or the other two forms of this family, so far as external appearance goes. But internally, in place of the buttress folds and main folds, there are double rows of semicircular or semilunar gills, each with nine to twelve villi on it; these villi are arranged in two rows, one each side of the crest of the gill. The villi vary in length, but are never longer than the gill is high. Each villus has two or three small setules on its rounded tip. The loops of the tracheae are in the tips of these villi as well as in the edge of the gill proper. Figures 12, 13 and 14 show these structures better than much description. Figure 15 is a contour map designed to show the formation of each double row. The longitudinal fold persists as a rounded protuberance, filled with fat, connecting the bases of the gills. I failed to find any tracheae in it.

In Anax junius there are usually seventeen or eighteen gills—for such the buttress folds have now become—in each single row. This is the lowest number found in the Aeschninae: Aeschna has twenty-five, Boyeria and Basiaeschna have about twenty-four each, Anax seventeen to nineteen. In one European species of Anax, as shown by Suckow ('28), there are sixteen to a row.

The structure of each gill corresponds to that of the buttress folds of other Aeschninae. There is fat in the base, through which the tracheae pass as they branch, and two epithelial cushions, not oblique but transverse in position, are on the base of each gill.

This genus has great historical interest, as it contains the 'Aeschna grandis' of Cuvier, Suckow, Dufour, Roster, and others. Except for detail differences in the placing of setules on the villi, variation in the number of gills to a row up to twenty-four, and variation in the number of villi to a gill, their figures and descriptions agree closely with what I found in A. junius.

Roster ('85) shows an Italian form of Anax that has twenty-two gills to a row and on each gill eighteen villi constricted at the base and bearing a crown of setules at the tip; the other forms have villi more like A. junius. Ris ('13) finds in Anax imperator gills and villi identical, so far as his description goes, with those of A. junius.

There are certain forms mentioned in the literature of the subject, and labeled 'Aeschna,' which deserve some comment. Dufour ('52) shows a form with semicircular or reniform gills, but lacking villi. I am inclined to think that this is some species of Aeschna, sensu moderno, or a closely allied genus, in which the longitudinal folds were missed; this overlooking of one or another of the sets of folds has been common. Miss Poletajev ('80) ,working on a Russian 'Aeschna grandis,' found gills substantially the same as I figure for buttress folds in Basiaeschna, but without any longitudinal fold. Here again I feel sure that something has been overlooked, but I do not know in what genus to place the form described. Ris ('13) assigns gills of this sort to Brachytron hafniense. Sadones ('95), working histologically, reports for an unknown species of Aeschna simple gills with two cushions; this is also an obvious case of omission to see some feature, and the form may be any Aeschnine.

D. Subfamily Gomphinae

In this subfamily I discuss first the rectum of the small nymph of Lanthus, and then four of the typical large burrowers. These latter show great similarity to each other, and connect with the Aeschnine series ending in Anax junius, but Lanthus stands alone in having what is in many ways the most remarkable rectum of any anisopteran form I have studied.

The rectum of the nymph of Lanthus parvulus (figs. 46 to 48) is smaller than any other rectum in the suborder, and is not over half as long and half as wide as the average. It is perfectly cylindrical, rounded at the anterior end, and tapering suddenly to the long anal canal. The distribution of tracheae to the dorsal side of the rectum is very much as in Cordulegaster, but the branches orginate as three large branches only, cephalad of the

postdorsal trachea. The three primary branches divide at once, as in the form referred to. The last branch is unusually large; the three branches are closer together than in any other member of the family and are separated far from the postdorsal trachea; in this the condition approaches that found in the Libellulidae. Figure 46 indicates this. The branching on the ventral side of the rectum is more highly specialized than in any other form in either family. The postventral trachea, the ten branches, and the trachea passing to the Malpighian tubules at the cephalic end of the small intestine, are all fused together for a short distance from the point where they leave the ventral trunk. They then separate suddenly; there is no dichotomy, but this big trachea suddenly breaks up into a fan of radiating branches, the most caudal of which is the postdorsal and the most cephalic of which passes to the Malpighian tubules. Even the ventral tracheation of Pantala flavescens, the extreme form of the Libellulidae, does not equal this. Figure 47 indicates the structures described.

The gill bases are in the usual six double rows, three on the ventral and three on the dorsal side. The gill bases are oval, set at an angle of only 20° from the transverse position. There are fifteen of the bases in each single row.

The interior of the rectum of Lanthus represents the greatest perfection of the longitudinal fold as a respiratory mechanism. The six longitudinal folds, each in the middle of a double row and homologous with those of the Aeschninae, are exceedingly high in proportion to the size of the rectum. They are smooth on the edge; their tracheation is as in the aeschnid forms that I have described. The buttress folds are exceedingly small and have only a slight connection at their proximal corner with the longitudinal fold. Their shape—elongate and pointed—is not unlike that of some libellulid gills. I was not able to detect the fat in their bases save from the outside of the rectum; and hence I cannot figure its extent. There appear to be the usual cushions on each side of the buttress fold. There are fifteen of these folds in each row, and the adjacent rows alternate in the usual manner. The longitudinal folds are smoothly rounded at each end of the rectum (fig. 48).

From this peculiar form, which would seem to be the climax of the series comprising most of the Aeschninae, we can turn to those more typical of the Gomphinae and allied in rectal structure to Anax. Four forms are here to be discussed.

In Hagenius brevistylis (figs. 17 and 18) the tracheation is intermediate between the few large dividing branches of Cordulegaster and the perfectly even series of branches of Aeschna. Each of the primary branches divides less often than in the forms thus far discussed, since each row has only twelve to fourteen gills. The gill bases are arranged as in the Aeschninae. Figure 7 shows the musculature of this species, which is identical with that of all other anisopteran recta. The anal canal is more marked than in the Aeschninae and Cordulegaster, and, as in all Gomphinae, is a definite tube supplied by a branch from each postdorsal trachea.

Within the rectum is a set of gills much like those of Anax. The single rows are not so definitely paired, and I was unable to find any remnant of the longitudinal fold connecting the gill bases. Each gill base, set at the same angle as in Anax, is oblong in shape, with the longer edge as the base. The ends are slightly diminished, and the gill base is triangular in cross section, with a sharp edge. On this edge are eleven or twelve villi. The villi are twice as long as in Anax, and all of the tracheal loops are in their tips; the villi are covered with setules (fig. 18). In the base of each gill is a mass of fat, and an elongate cushion occupies the greater part of each side of the base. The villi are on the crest of the base. The tracheation of each gill is from one primary or secondary branch, as in Cordulegaster; the same arrangement of two branches entering a gill is found here as in other nymphs.

Ophiogomphus carolus (figs. 19 to 21) show more typical Gomphine features in its rectum. The tracheation follows the aeschnine pattern, and is not to be distinguished from that of Gomphus (fig. 16). The gill bases are larger than in any form hitherto described, and are no longer in alternation but subopposite in adjacent rows. They are oval in outline and form twelve evenly spaced single rows, set at the angle of the aesch-

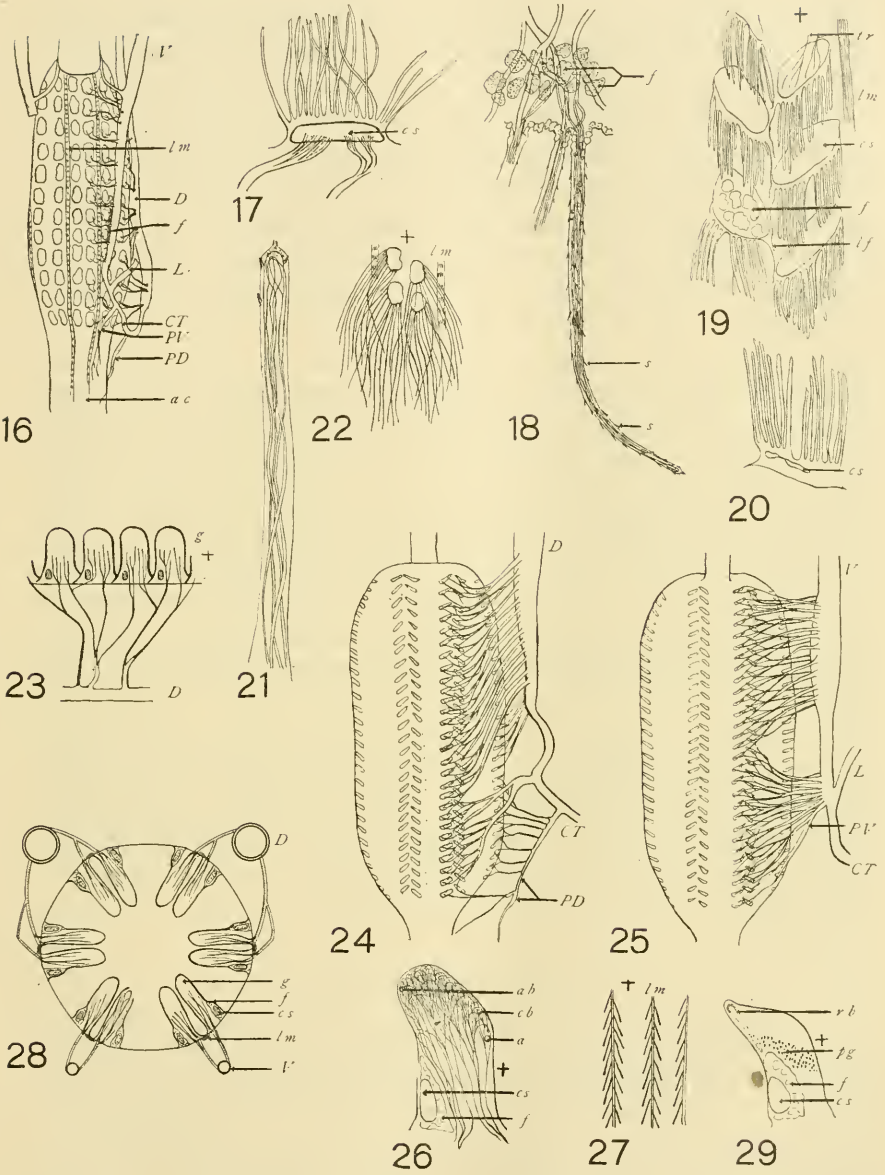
nine gill bases 45° from the transverse line. . Most typically gomphine is the way in which the tracheae pass into the gill bases of this form: they do not enter in the center, but pass in along the two edges.

Within the rectum the distribution of villi corresponds to this tracheation. The villi of each gill are clustered in a row along one curving side of the gill base; this is the side pointing caudad and towards the muscle band. The cushion on the cephalic side of the gill, compared to that of Hagenius, has become enlarged and flattened to an oval patch, while that on the caudal side is much reduced. The fat in the gill is all under the cephalic cushion. The villi do not taper and are the longest as yet seen; they bear a single seta on the conical tip and one or two on the sides near the tip.

Ophigomphus has a remnant of the longitudinal fold of the Aeschninae in the form of a ridge connecting the raised caudal edges of two rows of gills. Figure 19 shows this; figure 20 shows the caudal aspect of a gill, and figure 21 a villus.

Gomphus has a rectum essentially the same as in Ophiogomphus. The regularity of distribution of tracheal branches is here perhaps a little more perfect, but the two recta are not distinguishable externally. The appearance of the gill bases is shown in figure 16. In this genus I have dissected the two species, Gomphus descriptus (figs. 16 and 21) and G. villosipes. I find no

Fig. 16 Ventral view of rectum of Gomphus descriptus.
Fig. 17 Gill of Hagenius brevistylis.
Fig. 18 Villus and tracheation from gill of same.
Fig. 19 Cephalic aspect of gills of Ophiogomphus carolus, showing also arrangement of parts on inner surface of rectum.
Fig. 20 Caudal aspect of gill of same, villi lifted up.
Fig. 21 Villus of same.
Fig. 22 Gills of Gomphus descriptus.
Fig. 23 Distribution of tracheae to gills in Leucorhinia intacta.
Fig. 24 Dorsal view of rectum of Plathemis lydia.
Fig. 25 Ventral view of same.
Fig. 26 Gill of same.
Fig. 27 Arrangement of gills of same.
Fig. 28 Schematic cross-section of rectum of *Libellula* sp. (From a MS. drawing by Miss E. Andrews.)
Fig. 29 Gill of Didymops transversa.



(*For abbreviations see page 322.*)

difference between them save that the latter has a longer anal canal and hence a larger branch of the postdorsal supplying it. Otherwise the description of G. descriptus will fit both species.

The number of gill bases to a row in this form is twelve—the lowest number in any form I have studied. The gills are alike throughout the rectum. The gill bases are very nearly rectangular or oval, with the long axis pointing directly in a sagittal direction; they are subopposite, as in the last genus. The tracheal branches enter the gills at the same points as in Ophiogomphus.

Internally, the rectum of this form bears a mass of villi so long as to give a complete velvety covering to the walls of the chamber. In no other form is this so fully the case. The gills bases are mere pads of fat under an epithelial cushion, at the caudal border of which the long villi, exactly like those in Ophiogomphus, are attached. There are ten to twelve villi to a gill, and such of them as are not on the caudal edge are on the rounding corner of the gill nearest the longitudinal muscles. The caudal border of the gill is not in the least raised, nor has it any cushion caudal to the villi or any trace of longitudinal fold (fig. 22).

Ris ('13) describes in Onychogomphus forcipatus and Gomphus pulchellus, folds similar to those in Lanthus, but covered with villi. He also describes in detail a villus of Gomphus, finding its tracheation a little different from what I figure. He claims to find one very large trachea and several thin ones in each villus. I find no such differentiation.

II. FAMILY LIBELLULIDAE

In this family there are no such differences between the recta of the various subfamilies and genera as are found in the Aeschnidae. The agreement in structure in the three subfamilies is very great. The description of the rectum of Plathemis lydia serves for the present subfamily. It is an exceedingly common form and is in every way typical.

A. The Branchial Chamber of Plathemis lydia (figs. 24 to 27)

Scott ('05), studying the general tracheation of this nymph, described the whole respiratory system, but did not go into certain details of the rectum as far as necessary for this study.

Opening this short, stubby nymph discloses in the abdomen a brush of tracheae much closer together than those of Cordulegaster. The branches from the dorsal and ventral trunks alike are crowded close together and the trunks taper abruptly at the point where the branches arise. Eleven branches are given off from the trunk to the dorsal rows of gills, and a similar set to those situated laterally. Caudad of this bunch of branches, each dorsal trunk is free of branches for a space, and then gives off a brach, larger than the others, which passes to seven or eight gills at the caudal end of each of the four dorsalmost single rows. The postdorsal is quite as in the Aeschnidae. The ventral trunk has a similar bare place caudal to a bunch of branches, and then gives off a close group of five or six branches and the postventral trachea.

One feature of the rectum is very different from the aeschnid structure. The double rows of gill bases have apparently shifted in position. Three are to the right, three to the left of the median line. The branches from one side of the dorsal trunk do not supply halves of adjacent rows or share in supplying a median row. The branches from each side of the dorsal trunk and those from each ventral trunk pass to a particular double row of gill bases. Most of the branches divide once before they enter the bases, and the halves of a base are supplied from different branches. Figure 23, a teased preparation from an allied genus, shows this last feature. Figures 24 and 25 show the ventral and dorsal tracheation and the arrangement of gill bases. Thus the libellulid double rows are in no way homologous with those of the Aeschnidae; the single rows are homologous throughout both families.

The gill bases stand at an angle of about 40° from transverse, pointing caudad rather than cephalad, owing to the position of the double rows and the gills in them. The muscles of the rec-

tum are exactly as in all the forms hitherto described, but the shift of the gills brings each longitudinal band in the middle of a double row.

Internally, the rectum bears six double rows of gills, three on the right side and three on the left. The gills themselves are elongate protuberances, flat, with rounded ends, and undercut on one edge, as shown in figure 26. The arrangement of cushions and fat differs essentially from that in the Aeschnidae. There is but one cushion, which is on the cephalic side of the gill. The cushion is ovate in form, and overlies the mass of fat, which forms a triangle at the base of the caudal edge of the gill. Both cushions and fat are less prominent than in any aeschnid form. The tracheation of the gill does not in any way differ from that of the aeschnid buttress fold except that the tracheae do not pass through the fat in the base of each gill, but cephalad of it.

The gills are not set at the same angle as their bases. They stand about 15° from the sagittal line, and those of each row overlap like tiles. There are twenty-eight gills in most of the single rows. Figure 27 shows the arrangement of the gills.

On each gill three buttons, pads, or tubercles are found near the distal rounded margin. The most distal one is on the caudal side, the other two on the cephalic side of the gills. Between these and the thicker parts of the gill at the base, the water has space to circulate around the parts of the gill filled with tracheal loops and fine tracheoles (fig. 26).

The cross-section of the rectum of the allied genus Libellula (fig. 28, from Miss Andrews' MS.), shows some of the relations of the parts quite clearly, and indicates well the differences from aeschnids. The gills are of the same size in all parts of the rectum; in only a few specimens those in the cental part were a little larger than the others. There is no anal canal, the rectum tapering quickly and directly to the anal valve.

B. Subfamily Macromiinae

In this subfamily the only species available was Didymops transversa. In all features, save the shape of the gills, this agreed with the structure in Plathemis. Figure 29 shows the gill; it is sharp at the tip, the point being directed caudad. There are no buttons on the gills, but a ridge on the caudal side leads down from a bulbous projection near the tip of the gill. There are thirty gills in each single row. Tracheation, gill placement, fat, cushions, are all as in Plathemis.

C. Subfamily Cordulinae

The forms in this subfamily also show little difference from Plathemis or from each other.

In Dorocordulia libera (figs. 32 and 33) the tracheation is almost identical with that of Plathemis, but is somewhat less concentrated ventrally. The gills number thirty to a row. Figure 32, a typical gill; figure 33 is one of the six most caudal gills of each row. The gills at the caudal end are twice as high and wide as those at the cephalic end of the rectum; the sizes grade evenly along the rectum.

In Tetragoneuria cynosura the external features of the rectum are not distinguishable in any way from those of Plathemis. The interior of the rectum is also very similar to that of Plathemis, save that the button on the caudal edge of the gill is absent and the gills are slightly truncate at the tip. The most caudal five gills are a little smaller than the others, which are all alike in size.

The rectum of Helocordulia uhleri agrees remarkably with that of Dorocordulia libera in most respects, the only difference being that all of the gills are of the same shape as the most caudal of Dorocordulia. They grade in size, the most cephalic ones being slightly the smaller.

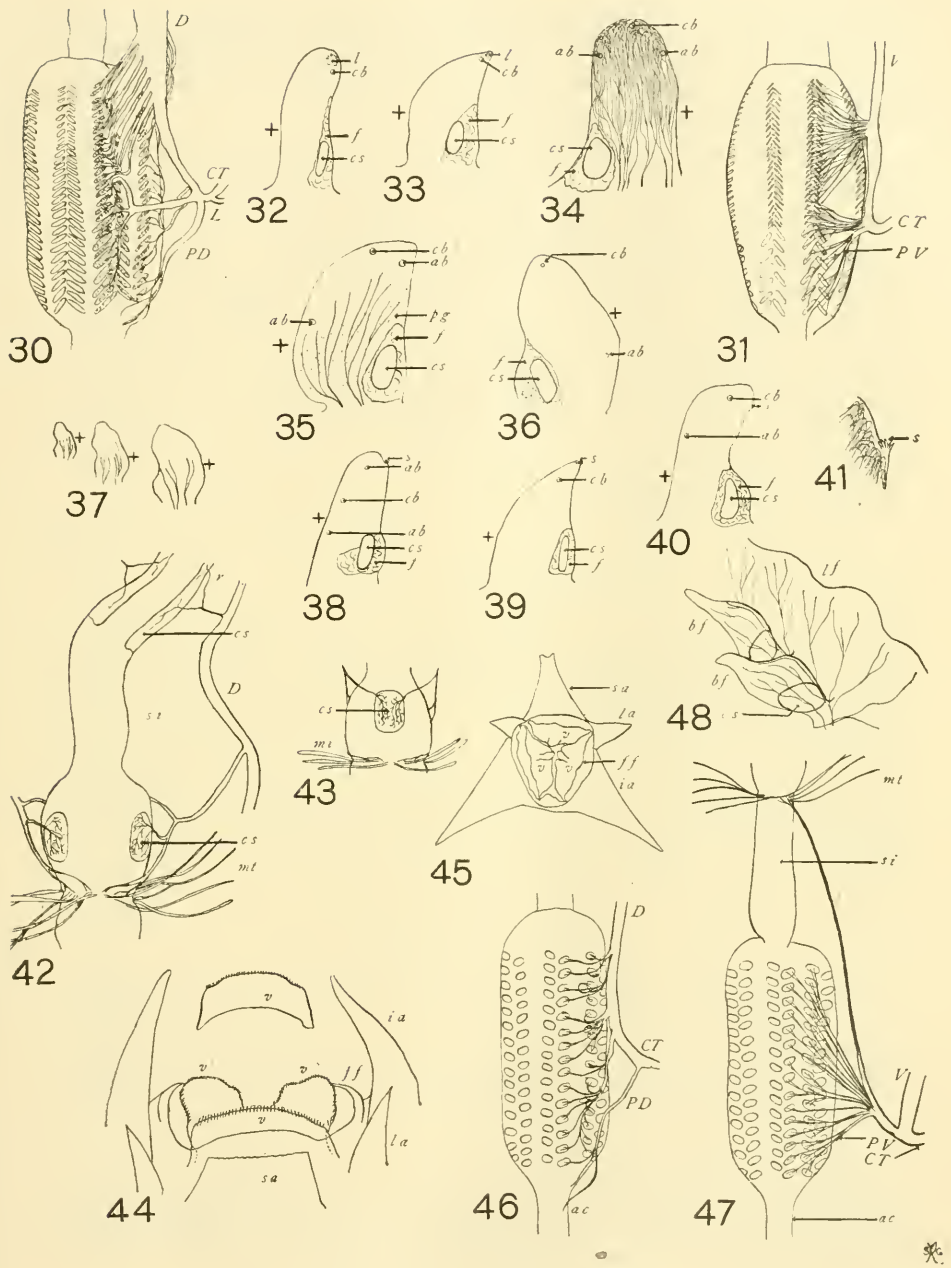
Miss Poletajev ('80) figures for a species of Epitheca some gills which do not agree with any cordulids that I have seen. They are shown as obovate plates, fastened at a point. Ris ('13) describes gills in Cordulia aenea identical with those in Plathemis.

D. Subfamily Libellulinae

The nymphs of this family are, as a whole, as much alike as in the other two subfamilies, the agreement of the several species of Libellula with conditions found in Plathemis is noticeable. But a number of genera, including Pantala and Tramea (figs. 30 and 31), show a distribution of tracheal branches slightly different from that found in Plathemis. The gills of Leucorhinia intacta have the form shown in figure 34. The tracheal distribution to the gills in this species is shown in figure 23. There are eighteen gills in each row and they are all alike in size; their tracheation is marked by an unusually parallel arrangement of tracheoles in the gill body. Sympetrum obtrusum (fig. 35) offers somewhat of a different form of gill. The shape is here like a quarter of a circle, or perhaps more nearly a quarter of an oval, with the straight edge caudal. The cushion is unusually small.

The rectums of Pantala flavescens and Tramea carolina are specialized in that the gills at the two ends of the rectum have not the same size and shape. They agree in tracheation, and show the extreme type of bunching of branches. I figure the

Fig. 30 Dorsal view of rectum of Tramea carolina.
Fig. 31 Ventral view of rectum of Pantala flavescens.
Fig. 32 Typical gill of Dorocordulia libera.
Fig. 33 Caudalmost gill of same.
Fig. 34 Gill of Leucorhinia intacta.
Fig. 35 Gill of Sympetrum obtrusum.
Fig. 36 Gill of Tramea carolina.
Fig. 37 Gradation in size of gills of same; largest gills are at caudal end of rectum.
Fig. 38 Gill from cephalic end of rectum of Pantala flavescens.
Fig. 39 Gill from middle of rectum of same.
Fig. 40 Gill from caudal end of rectum of same.
Fig. 41 Setules from tip of gill of same.
Fig. 42 Dorsal view of rectum and small intestine of Argia putrida.
Fig. 43 Ventral view of intestinal ampulla of same.
Fig. 44 Dorsal view of anal valve of Plathemis lydia, with superior appendage cut away, a single lobe is also shown separately.
Fig. 45 Caudal aspect of anal valve of Basiaeschna janata.
Fig. 46 Dorsal aspect of rectum of Lanthus parvulus.
Fig. 47 Ventral aspect of same.
Fig. 48 Longitudinal and buttress folds of same.



(For abbreviations see page 322.)

dorsal view of the rectum of Tramea and the ventral view for Pantala. The large last branch, just cephalad to the postdorsal trachea, is a feature here carried far beyond its extent in the other members of the family.

Tramea carolina (figs. 36 and 37) has its gills evenly graded in size, those at the caudal end of the rectum being two and one-half times the size of those at the cephalic end in every dimension. The gill bases show this markedly on the outside of the rectum. There are thirty gills in each single row. Each gill is emarginate along the caudal edge and slightly so on the cephalodistal margin. The epithelial cushion is unusually far from the caudal edge, and is rather quadrangular as well as somewhat oblique in position. Figures 36 and 37 show the features of these gills. In all other respects this rectum agrees with that of Plathemis.

In Pantala flavescens (figs. 31 and 38 to 41) the gills are specialized in a different manner. Of the thirty gills in each row, the last six are twice as high and wide as any of the others. The gill bases change suddenly in size at the same point (fig. 31). The twenty-four cephalic gills are all of the same size, but those at the cephalic end are of the same shape as the cephalic gills of Dorocordulia, while the middle fifteen have blunt tops and are much like the gills of Plathemis. Figure 41 shows in details a feature found in this genus only: a bunch of five to seven setules near the distal end of the caudal edge. Other features of this rectum are as in Plathemis.

THE ANAL VALVE

The perfected respiratory organ of the anisopteran nymph has its means of excluding both harmful liquids and solid particles which might cause injury. To Scott ('05) belongs the credit of first describing this remarkable anal valve; Sadones ('95) seems to have noticed its parts, but not their mutual relations or their function.

The anal valve of Plathemis lydia was described by Scott as consisting of three meniscus-shaped chitinous lobes projecting into the lumen of the rectum at its extreme caudal end, just

where it emerges among the appendages of the abdomen. The three lobes are hinged at their base, and the one on the dorsal border of the anus laps over the two lateroventral lobes when closed. My own observation corroborates this, and figure 44 shows the appearance from the dorsal side with one appendage removed. The edge of each valve-flap bears a row of fine hairs; the three valves fit together perfectly and seem to make a water-tight joint. Outside of each lobe there is, in my material, a similar set of fleshy folds, which seem to provide bases on which the lobes rock. The action of the valve is often conspicuous: it opens and shuts with every suction of water in some nymphs.

The anal valve is present in all the nymphs which I have studied. In the Aeschnidae the lobes are less heavily chitinized; in Basiaeschne, as figure 45 indicates, they are rather membranous and wrinkle when holding the rectum closed. In Gomphus they are more chitinous.

THE ZYGOPTERAN RECTUM

Dufour ('52) states that Calopteryx virgo nymphs have three richly tracheated cushions, free at the caudal end, and hanging into the lumen of the rectum. This is denied by some, affirmed by others. Dewitz ('90) claimed that rectal gills are present in the Agrionidae.

My own work covers five species of this suborder, chosen from a wide range within it. The recta of four of the forms, Lestes forcipata, Argia putrida, Enallagma hageni, and Cora chirripa, as well as Mecistogaster modestus, studied by Calvert ('11), may be dismissed with the statement that they have only the usual longitudinal rectal glands, similar to those in other orders of insects. The number of these is three or six. There is no tracheation beyond a single small branch from each dorsal trunk; nothing approaching a breathing mechanism. These recta are not in any way enlarged.

The rectum of Calopteryx maculata is expanded into a globe and has three large pads, one middorsal and the other two lateroventral. From the sections made by Dr. Calvert, which he was kind enough to let me use, it is evident that these are

loose bags filled with fatty cells and tracheae. The tracheae passing to these fatty plates are branches from the dorsal trunks only, but they are larger than those in the other Zygoptera discussed, and branch extensively on reaching the bags.

Whether or not this be a respiratory organ I cannot say. It comes the closest of any form outside the Anisoptera to the longitudinal folds which I found in so many Aeschnidae. The presence of fat, tracheae, and a thin epithelium over all comes close to some of the details of anisopteran gill structure. The rectum of Calopteryx resembles the intestinal ampulla of Agria, shown in figures 42 and 43.

THE INTESTINAL AMPULLA OF ZYGOPTERA

Careful dissection of the abdomen of a nymph of Argia putrida reveals the structures shown in figure 42. The rectum, as previously explained, offers nothing of interest. The end of the intestine, just caudad of the Malpighian tubules, is expanded into a large globular ampulla, on whose sides are three fatty plates, to which tracheae pass. Two of the plates are laterodorsal in position, and the third one midventral. Figure 43 shows the ventral aspect of the ampulla.

Each of these plates is supplied by a branch from the trachea which passes to the Malphigian tubules; two branches, one from each side, go to the midventral plate. The surface of each plate is covered with a thin, tough epithelium, much like that of the anisopteran respiratory folds or gills. Beneath this is the mass of fat, among whose cells the tracheal branches pass. I was not able to determine whether or not the tracheae loop here as in the anisopteran respiratory organs.

In Calopteryx maculata the same structure is present. I have not observed this region in other species. The function of this organ is unknown to me. I am inclined to grant it some respiratory use, in view of the fact, well known and observed by me, that Argia and other Zygopteran nymphs can live for days without the external caudal gills.

PHYSIOLOGICAL CONSIDERATIONS

Dissolved in the water which enters the rectum are a number of gases, each exerting its partial pressure independent of the others. To some, as oxygen and carbon dioxide, the lining of the rectum is a permeable membrane; they pass through it. Dewitz ('90) has shown that these gases pass through chitin. If the pressure of the gas in question be greater in the air within the tracheae, no inward passage will take place, but the reverse. The question of whether or not one side of the rectal wall differs from the other in having the gas in the form of a solution or a free gas, does not make any difference here, for the wall is impervious to the solvent.

As the activities of the nymph use up a part of the oxygen in the air within the tracheae, more of this gas will diffuse in through the gills from the amount present in the water, whose pressure is thus greater than that of the oxygen within the tracheae. Similarly, the activities of the nymph soon cause enough carbon dioxide to accumulate within the tracheae to cause diffusion outwards through the walls of the gills.

The question of circulation of air in the tracheal system does not seem either important or difficult to me. The active contraction of the abdomen in the working of the rectum for swimming or respiration ought certainly to produce a considerable amount of movement of air in the main tracheal trunks. Given this, the immense amount of area for diffusion which the gills provide certainly suffices for gas exchange, with only the diffusion of the gases within the tracheae to bring carbon dioxide to the seat of exchange and carry away the oxygen. What eddy currents may exist and aid this, I cannot say; it seems likely that some do exist.

SUMMARY

1. The rectum in all anisopteran nymphs possesses six longitudinal rows of respiratory structures, each of which is divided symmetrically into halves. The six rows are situated, three on the dorsal and three on the ventral walls in the Aeschnidae,

and three on the right and three on the left wall in the Libellulidae. In either case they are evenly spaced around the rectum. The single rows are all homologous, but the grouping of them in double rows is different in the two families.

2. The rectum receives numerous branches from the dorsal and ventral tracheal trunks: those from the dorsal trunks supply the dorsal two-thirds of the rectum; those from the ventral trunks, the ventral third only.

3. In the Aeschnidae there is an anal canal extending caudad of the rectum.

4. Each half of one of the six longitudinal rows of respiratory structures consists of a series of twelve to thirty gills or folds, which may bear villi distally. In the base of each gill or fold is a mass of fat, and on the surface of it one or more thickened epithelial cushions; the distal end of the gill or fold or villus upon this is filled with loops of tracheae and is the part functional in respiration. Each gill or fold receives two tracheal branches.

5. In the Aeschninae, Cordulegaster, and Lanthus there is also a tracheae longitudinal fold extending between the closely adjacent halves of each row of respiratory structures.

6. In the Gomphinae (except Lanthus) and in Anax, the gills bear villi along the edge, and these are the respiratory structures. The number of gills in these forms is at its lowest.

7. In the Libellulidae there are no villi and no longitudinal folds, and the longitudinal rows are arranged in pairs in positions intermediate between those they occupy in the Aeschnidae. Each half row consists of about thirty linguiform flattened gills, extending lengthwise of the rectum, or nearly so, and separate from each other. There are no epithelial cushions on the cephalic face of the gills, and the fat is restricted to the most caudal angle of the gill.

8. The tracheae which pass to the rectum are evenly spaced along the trunks in the Aeschnidae, except in Cordulegaster, where there are four large branches which divide at once. In the Libellulidae the branches arise very close together and distinct from the tracheae which continue the trunks caudad. The

branching in Lanthus is markedly different from that in any other form studied.

9. There is a three-lobate valve at the caudal end of the rectum.

10. The Zygoptera show no gill chamber, but have an enlarged ampulla just caudad of the Malpighian tubules. In Calopteryx the rectum is enlarged to form a second ampulla, but it does not resemble the anisopteran rectum.

11. The following table will show the number of respiratory parts found in each rectum. The figures are approximate, except for the longitudinal folds; they are based on average numbers of gills from several rows and average numbers of villi from many gills.

GENUS	BUTTRESS FOLDS	GILLS	VILLI	LONGITUDINAL OLDS
Cordulegaster	216			6
Aeschna	280			6
Basiaeschna	280			6
Boyeria	280			6
Anax (A. junius)		216	1950	
Lanthus	180			6
Hagenius		160[1]	1900	
Ophiogomphus		(240)[1]	4800	
Gomphus		(160)[1]	2900	
Didymops		360		
Epicordulia		360		
Dorocordulia		360		
Tetragoneuria		350		
Helocordulia		370		
Leucorhinia		216		
Sympetrum		200		
Pachydiplax		360		
Libellula		340		
Plathemis		340		
Tramea		360		
Pantala		360		

[1] Morphologically present, not functional in respiration.

Postscript

Since this paper was completed, J. E. Tillyard's article, "A study of the rectal breathing apparatus in the larvae of Anisopterid dragonflies," has appeared (Trans. Linn. Socy. London, Zool., vol. 33, 1916). While the results obtained independently by Tillyard and myself are similar, the forms studied are so different, that there is no undesirable duplication.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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A PHYSIOLOGICAL STUDY OF THE ANATOMY OF THE EYE AND ITS ACCESSORY PARTS OF THE ENGLISH SPARROW (PASSER DOMESTICUS)

JAMES ROLLIN SLONAKER

From Stanford University, California

SEVENTY-FIVE FIGURES

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INTRODUCTION

The eye of the bird, owing to its keen power of sight, its great range of accommodation, and its relatively large size, is an especially interesting object to study. The purpose of this paper is to bring together in as compact a form as possible: 1. A complete morphological description of the various parts of the eye and its accessory structures. 2. The functions of these parts. It is also intended to serve as a basis of comparison for an extended study of the eyes of the birds of the world which Dr. Casey A. Wood, of Chicago, and the author have under way.

In this connection I desire to thank my colleague, Dr. Wood, for kind assistance rendered during the preparation of this article.

The English sparrow (Passer domesticus) has been chosen because of its almost cosmopolitan distribution, its abundance, and the ease with which it can be secured.

Adult sparrows were secured alive by means of traps and were confined in cages until needed. An abundance of fresh material was thus secured. This also enabled one to make various observations in regard to the movements of the eyes and lids and the angle of vision. To test the power of convergence, one eye was removed from several birds. These experiments will be described later.

The eyes were removed immediately after death and hardened in Perenyi's fluid and imbedded in celloidin. Serial sections were made and stained in haematoxylin and eosin. Perenyi's fluid preserved the retina in the most perfect condition of any hardening fluids tried, and at the same time decalcified the bone wherever present. In some cases the whole head was preserved and sectioned to show the relations of the various parts. Sections were made in both horizontal and vertical planes parallel to the axis of vision.

To facilitate the dissection of the blood supply to the eye and accessory parts fresh specimens were used. The arteries were injected with carmin-gelatin and the veins with blue. The binocular dissecting microscope was used in the dissection of the various structures. Camera-lucida drawings were made wherever possible. All other drawings were made to scale.

THE EYEBALL

The eye of the sparrow, as in all birds, is relatively very large. When the eye is wide open, the interpalpebral space is practically a circle and is entirely filled by the cornea, none of the sclerotic being visible. This condition prevails during the time that the bird is awake, as the true lids take no part in bathing the front of the eye with the lacrimal secretions. The shape and size of the eye of the sparrow are quite different from that of

man. When the relative size of the eye and the brain of the bird and man are compared a great difference is noted. This is graphically shown by Pütter ('12). Leuckart ('76) finds that the weight of both eyes of some birds is greater than their brain weight. I have found this to be true in a young eave swallow (Chelidon erythrogaster), just able to fly, whose brain weighed .521 gram and the two eyes .625 gram. The brain weight thus forms a ratio to the combined weight of the eyes of approximately 5 to 6. In the California blue jay (Aphelocoma californica) I have found the brain weighs 3.433 grams and the two eyes 2.613 grams. In the sparrow I have also found that the reverse is true, for the brain weighs almost two and one-half times as much as the two eyes. The average weights are: brain, 1.0803 gram; the two eyes, .4455 gram, a ratio of brain weight to eye weight of a little more than 2.4 to 1. The weight of the human brain is many times the combined weight of the two eyes. Making use of Vierordt's ('93) tables of weights of these two organs, we find that the ratio of brain weight to that of the eyes is 51 to 1.

Since the lids cover the greater portion of the eye of the bird, its large size is not apparent until it is fully exposed. Figure 8 shows the skin and lids dissected loose and turned forward, exposing the eye. The equatorial diameter of the sparrow eye is 7.4 mm. and the axial diameter is 6.8 mm. The similar dimensions of the human eye are 24.5 mm. and 24 mm., respectively. The relative size of the sparrow and the human eye is diagrammatically shown in figure 1. The ratio of the radius of curvature of the cornea to that of the posterior part of the eye is approximately 1 to $2\frac{1}{4}$. Man shows a ratio of about 4 to 5.

The two eyes are so situated as to almost touch each other at the median plane (figs. 12, 18, and 19). The eye socket is very shallow. The eyeball, extrinsic muscles, blood-vessels, nerves, and glands practically fill the socket, leaving little room for adipose tissue. The bony portion of the anterior part of the orbit extends but slightly beyond the equator of the eye. The posterior portion, however, reaches almost to the junction of the sclerotic and cornea.

The axes of vision in the eye of a dead bird form an angle of about 65 degrees with the median plane. This is due to the lateral position of the eyes in the head (fig. 29, *AF*, plate 6). The angle formed by the axes of vision of the two eyes is 130°. With this wide angle it does not seem possible for binocular vision to exist along the axes of vision. In life the ability to reduce this angle is very marked. Owing to the very short nerve and the close fitting orbit, I do not think it possible for binocular vision to occur in which the fovea of each eye is involved. The experiments bearing on this will be described later.

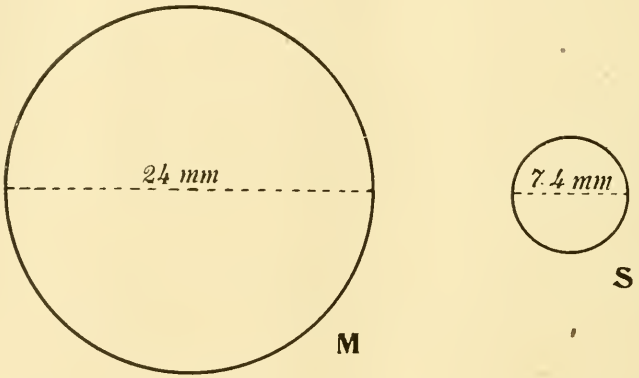


Fig. 1 Diagram showing the comparative size of the human and the English sparrow eye. × 2.

As in all vertebrates, the eye of the sparrow consists of three concentric layers which contain three refracting media. The three layers are: 1) the sclerotic, or supporting structure; 2) the chorioid, or vascular tunic, and 3) the retina, or nervous layer. The refracting media are: 1) the aqueous humor in front of the lens; 2) the crystalline lens, and 3) the vitreous humor behind the lens. Before dealing with these structures the following accessory parts will be described: *a*) the eyelids; *b*) the lacrimal apparatus; *c*) the extrinsic muscles; *d*) the blood supply to the eye; *e*) the nerve supply to the eye.

THE EYELIDS

The structures which protect the front of the eye of the sparrow consist of three parts: the upper lid, lower lid, and the nictitating membrane.

The upper lid is short, thick, and almost immovable. The lower lid is much longer, is thinner, and is capable of great movement; in fact, the closure of the eye is due mainly to the movement of the lower lid. When the lids are closed they meet well above the pupil (figs. 2 and 9).

The margin of the two lids is much thickened, very firm, and has a horny appearance. These thickened edges are segmented

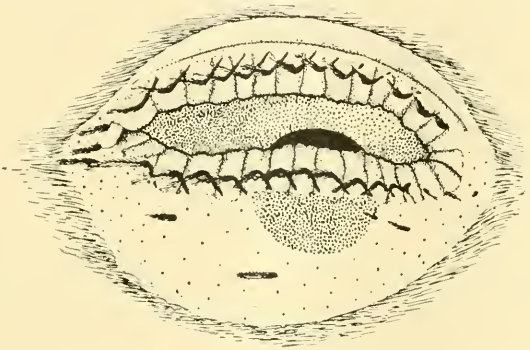


Fig. 2 Enlarged semidiagrammatic drawing of the right eye of the sparrow after death, showing the margins of the lids terminating in a thickened convoluted ring. A small tuft-like feather arises from the base of each of these folds with the exception of a few at the anterior and posterior angles.

into sausage-like portions of irregular size and shape. Figure 3, representing the right eye of the sparrow drawn from life, shows these marginal folds and the general external appearance of the lids. These folds are more or less partially divided so that the number is not constant; the upper lid has from 17 to 19 and the lower lid about 18 divisions. Since the edges are firm and stiff, it is readily seen that the function of these folds is to allow the margins of the lids to bend so as to meet each other at all parts when the eye is closed. When the lids are completely closed the junction does not form a straight line, but a slight curve upward.

There are no structures on the lids of the sparrow which correspond to the eyelashes in mammals. A narrow row of very small feathers, or plumules (*F*), which overlap in a shingle-like manner, lies just above the marginal folds of the upper lid. It extends from about 1 mm. above the anterior canthus (*Ac*) to the posterior canthus, where it is joined by a single row from the lower lid. The row on the lower lid differs from that on the upper in that the plumules are farther apart and are deflected downward and backward so that they scarcely overlap. The row on the upper lid lies closer to the marginal folds than does the lower, and, in the region directly above the pupil, somewhat overlaps them. The function of this upper row, I think, is to protect the eye somewhat from the bright light which might

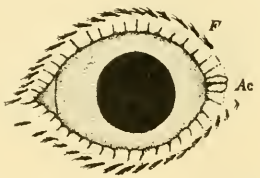


Fig. 3 Drawing from life of the right eye of the sparrow, showing the thickened convoluted margins of the lids and the shape of the interpalpebral space. × 5.

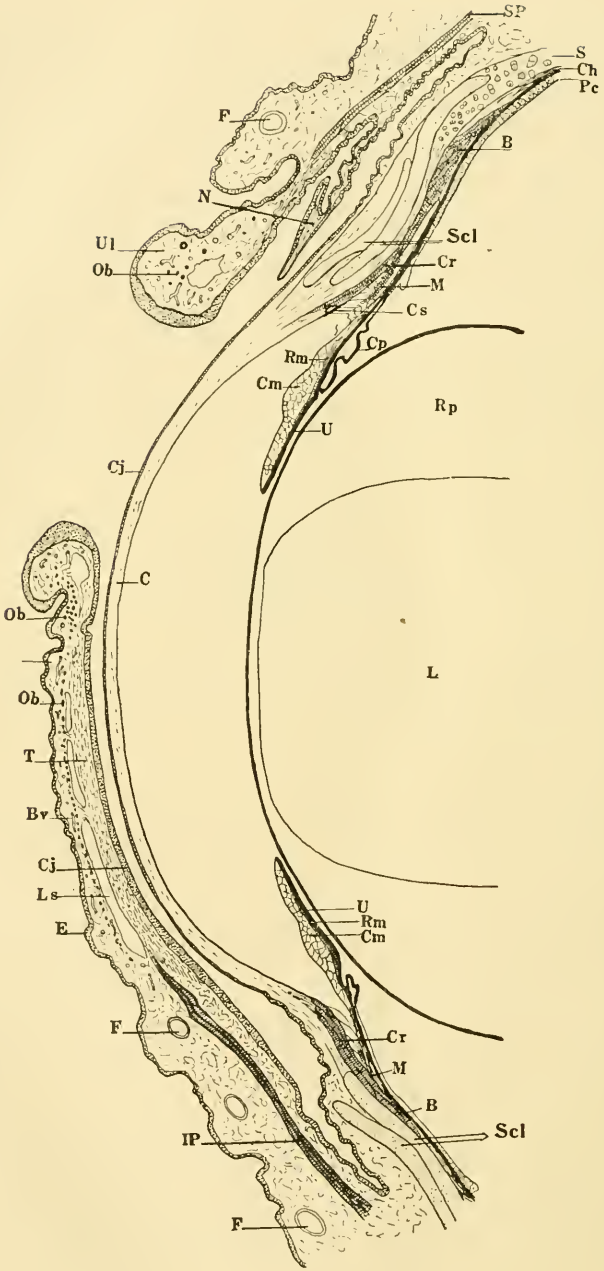
enter it from above. There is a narrow space devoid of feathers just above this marginal row of plumules on the upper lid, and above this bare area are the feathers covering the head. These true feathers of the head overhang the eye so as to protect it from the rays of light from above. Besides this marginal row of plumules on the lower lid, there exist a few plumules scattered over a more or less wide area, which conforms in shape and size to the outline of the eye which lies under the skin. When the eyes are wide open these bare areas are scarcely noticeable unless one parts the feathers. When the lids are closed, however, they become more conspicuous, and this is more noticeable on the lower lid (figs. 2 and 9). When the lower lid is closed, its thin and translucent appearance is noticed. The pupil and the iris can be indistinctly seen through it.

The structure of the two lids is in general very similar. The upper lid is somewhat thicker than the lower. The thickness of the marginal folds varies from .324 mm. to .432 mm. The thinnest portion, which is just above the marginal folds, is from .144 mm. to .198 mm. in thickness. Corresponding measurements of the lower lid show the marginal folds to be from .243 mm. to .400 mm. and the thinnest place about the center of the tarsus-like plate is from .09 mm. to .27 mm.

The skin over the front of the lids is very thin and delicate. It becomes much thicker at the marginal folds and is thickest at the inner margins where it merges into the conjunctiva (fig. 4). Over this portion the basal cells are long and cylindrical and are covered by stratified epithelium. Numerous pigment cells are scattered among the epithelial cells of the free margins of the lids and are especially abundant in the long cylindrical cells where the transition to conjunctiva occurs (fig. 4, and plate 10, figs. 58, 59, 60, and plate 11, figs. 63, 67).

The conjunctiva is thickest near the margin of the lids. This thickened region extends for about 1 mm. from the margin. From this point on over the inner surface of the lids, the conjunctiva becomes rapidly thinner until at last it has a uniform thickness. In the region of the inferior fornix conjunctivae, this membrane, as well as the underlying connective tissue, is greatly folded and convoluted (plate 10, fig. 59). This convoluted appearance is scarcely noticeable in the superior fornix conjunctivae (fig. 58). This is what one would expect when the movement of the two lids is considered. The folds make it possible for the two lids to open and close without putting the conjunctiva on a stretch and injuring it. The conjunctiva not only lines the lids, but, at the fornices, is reflected over the whole of the front of the eyeball and completely envelopes the nictitating membrane (fig. 61, plate 10).

In the region of the inferior fornix the conjunctiva contains numerous goblet cells. Doenecke ('99) has demonstrated these goblet cells in both lids of the hen. In the sparrow I have found them only in the conjunctiva of the inferior fornix. They are equally numerous on both the bulbar and the palpebral portions.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They rapidly diminish in number as one leaves this region, and are wanting in the main portion of the lower lids and the balance of the conjunctival surface. The tarsal or Meibomian glands of mammals are entirely lacking in birds.

An approximatal oval plate, 2.43 mm. broad and 1.98 mm. in height, resembling the tarsal cartilage in the lid of man, occurs in the lower lid, but there is none in the upper. It covers the greater part of the extent of the lower lid (figs. 5 and 8). It is almost uniform in thickness over the greater portion with the exception of the edges which diminish quickly. A slight difference is noted in the thickness between the upper part and the lower, to which the inferior palpebral muscle is attached, the upper part being .073 mm. and the lower .099 mm. in thickness. This extra thickness evidently compensates for the strain due to the pull of the muscle. The whole plate is saucer-like, conforming to the curvature of the cornea. At first sight this tarsal-like plate resembles cartilage, but there are no cartilage cells present. It is composed of a firm and closely connected tissue mass. This plate lies immediately adjacent to the palpebral conjunctiva.

About midway between the two surfaces of the lids are large lymph spaces, more or less connected and crossed by connective-tissue bundles, blood-vessels, and nerves. These spaces extend from the marginal folds to the region of the distal attachment of the palpebral muscles. A vertical section through the lids shows the cross-section of a few muscle fibers, constituting the orbicularis muscle which serves to close the lids. Unlike all the other muscles of the eye, the orbicularis is composed of non-striated fibers.

Fig. 4 Enlarged semidiagrammatic drawing of a vertical section of the adult sparrow eye. *B*, Brücke's muscle; *Bv*, blood-vessels; *C*, cornea; *Ch*, chorioid; *Cj.*, conjunctiva; *Cm*, circular muscles of the iris; *Cp*, ciliary processes; *Cr*, Crampton's muscle; *Cs*, canal of Schlemm; *E*, epithelium; *F*, feather follicle; *IP*, inferior palpebral muscle; *L*, lenticular portion of the lens; *Ll*, lower lid; *Ls*, lymph spaces; *M*, Müller's muscle; *N*, nictitating membrane; *Ob*, orbicularis muscle fibers of the lid; *Pc*, pars ciliaris retinae; *Rm*, radial muscles of the iris; *Rp*, ring-like pad of the lens; S, sclera; *Scl*, scleral plates; *SP*, superior palpebral muscle; *T*, tarsus; *U*, uvea; *Ul*, upper lid.

The muscles which move the lids may be classed under two groups: 1) the levator palpebrae superioris and depressor palpebrae inferioris, which function in opening the eye; 2) The orbicularis palpebrarum, which closes the lids.

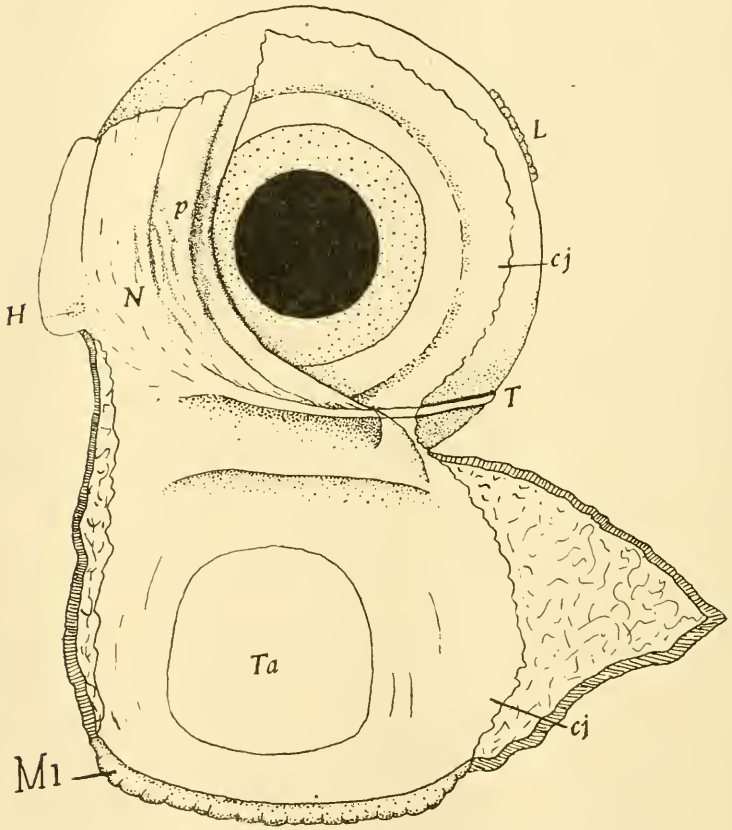


Fig. 5 Left eye of the sparrow with the lower lid dissected loose and turned down to show the opening of Harder's gland (*H*) beneath the nictitating membrane (*N*) and the attachment of the tendon (*T*) which moves the membrane. *Ml*, margin of lid; *cj*, conjunctiva; *L*, lachrymal gland; *p*, pigment portion of the nictitating membrane (shaded portion); *Ta*, outline of tarsus. × 8.

The superior and inferior palpebral muscles have their origins at the posterior part of the eye socket just back of the region of the origin of the rectus muscles. From this place they extend outward around the eye, above and below respectively, to their

insertions in the two lids. At their proximal end they are narrow, but as they extend outward they become gradually broader until they reach their insertion. In the other dimension they are thin and ribbon-like. The lower muscle is larger than the upper. The insertion of the inferior palpebral muscle is at the lower anterior surface of the tarsal-like plate. This muscle is somewhat larger about midway between its two attachments. The superior palpebral is more slender and has more tendon and less muscle tissue in its distal portion than the inferior. Its insertion is well down toward the marginal folds of the upper lid. No plate-like structure is found in this lid.

The orbicularis palpebrarum, as already mentioned, is composed of non-striated fibers, which are arranged approximately parallel to the margins of the lids. It functions in closing the lids.

One marked difference is noticed in the rapidity with which the lids are opened and closed. They open rapidly and close very slowly. This is due to the structure of the muscles involved in these two movements. The quick opening of the eye is often of urgent need to the bird in order to save its life from some danger while the closing is not so vital. This rapid opening can only be accomplished by striated muscle fibers.

When at rest the nictitating membrane, or third eyelid, is scarcely visible in the interpalpebral space. Even when it is pulled over the front of the eye, a correct idea of its size and shape is not obtained, as only a portion of it is exposed to view. When the lower lid is dissected loose and turned down, and the upper part of the eye is exposed, the full extent of the membrane is seen. Figure 5 shows it in the relaxed condition. It forms, with the eyeball, a pouch or bag at its lower anterior portion, into which the duct from Harder's gland opens. The secretion from this gland is thus poured under the nictitating membrane and directly on the front of the eye.

Numerous folds occur on the surface of the nictitating membrane. Sections show these to be largely superficial and confined almost wholly to the epithelial layer of the conjunctiva which envelops the membrane. They largely disappear when the membrane is extended over the eye.

The free margin of the nictitating membrane is composed of a stiff and firm band of dense connective tissue arranged so as to form a plait-like structure on the anterior surface at the free edge. This firm band I have called the marginal plait of the nictitating membrane. It thus forms on the anterior surface of

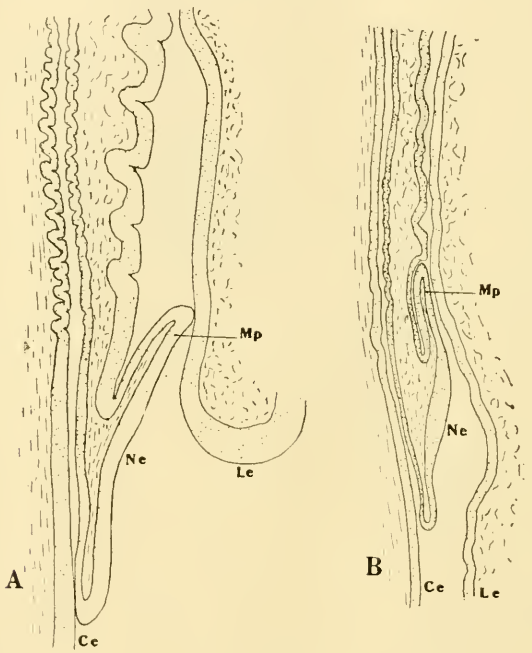


Fig. 6 Enlarged camera outline drawings, showing the marginal plait of the nictitating membrane extended (*A*) and compressed (*B*). *Ce*, conjunctival epithelium of the cornea; *Le*, epithelium of the lid; *Mp*, posterior edge of marginal plait; *Ne*, epithelium of nictitating membrane.

the third lid a scoop-like projection, extending anteriorly the entire length of the margin. A cross-section of this plait is shown in figure 6 and plate 10, figures 57, 58, 61. In cross-section the plait resembles the barb of a fish hook. The posterior margin, which corresponds to the free margin of the nictitating membrane, is thin and closely applied to the corneal surface. As the nictitating membrane is pulled over the front of the eye the margin of this plait is somewhat pressed downward against

the membrane (fig. 6, *B*), but, as the membrane is drawn forward to its resting position at the anterior angle of the eye, this plait springs or turns out and thus rubs against the lining of the lids (fig. 6, *A*).

The physiological significance of this arrangement is apparent. Harder's gland, opening into the sac between the nictitating membrane and eyeball, keeps the inner surface of this membrane well supplied with its secretion. When the membrane is swept over the front of the eye it copiously bathes its conjunctival surface. At the same time the thin edge allows detritus and excess of fluid which accumulated on this surface to flow on to the anterior surface of the nictitating membrane. The marginal plait, lying flat, allows this to occur more readily. As the membrane returns to its passive position, this scoop-like plait collects this excess of fluid and carries it forward between the lids and the nictitating membrane to the anterior canthus, where it escapes into the lacrimal canals.

The supporting structures of the nictitating membrane consist almost wholly of elastic fibers and connective tissue, interspersed with numerous blood-vessels, nerves, and lymph spaces. Numerous pigment cells occur in the middle portion of the marginal plait. Doenecke ('99), Fumagalli ('99), and Ellenberger ('06) have demonstrated the presence of smooth muscle fibers in the nictitating membrane, but I have not seen such tissue in the sparrow. The structures of the nictitating membrane are so arranged and so transparent that when the membrane is over the eye it can only slightly interfere with vision. This transparent quality, coupled with the extremely rapid movement with which this membrane is swept across the eye, leaves the field of vision clear practically all the time.

The tendon from the pyramidalis muscles which pulls the nictitating membrane over the eye is attached to the lower end of the marginal plait in the region of the inferior fornix. The downward and backward pull of this tendon not only moves the membrane, but also flattens the scoop-like marginal plait.

The upper end of the marginal plait is rather firmly attached to the eyeball, which prevents it being pulled away from that

portion. The movement of the free margin of the third eyelid, therefore, approaches that of a pendulum. This movement is accomplished in the following manner: the contraction of the pyramidalis and quadratus muscles (described elsewhere) draws the membrane over the eye, at the same time it puts the elastic tissue of the third lid on a stretch. On relaxation of these muscles the elastic fibers draw it quickly forward to its resting position. If smooth muscle fibers are present in this membrane as described by the authors mentioned above, owing to their slow action they can take no part in these extremely rapid movements.

THE LACRIMAL APPARATUS

The glands of the eye of the sparrow are two: the lacrimal gland proper and Harder's gland. There is a great difference in the size of these two glands.

The lacrimal gland is small, triangular, and flat (about 1.25 mm. across), and lies closely adjacent to the eyeball, slightly below the equator and temporal to the outer canthus of the eye (fig. 13, *Lg*). It is abundantly supplied with minute blood-vessels from the external ophthalmic artery and vein. It is innervated mainly by branches from the lacrimal nerve (fig. 27, *l*) soon after it leaves the inferior orbital branch of the superior maxillary nerve. It also receives some small twigs directly from the inferior orbital nerve. The gland thus lies in the angle formed by the superior orbital, inferior orbital, and lacrimal nerves (figs. 18 and 19, *lg*).

Its secretion, which is scanty, enters the conjunctival sac at the lower temporal portion of the lower lid, and is evidently used to lubricate this portion of the lower lid which is not reached by the nictitating membrane. According to Sardemann ('87), a single wide duct leads from this gland to the lower lid.

Harder's gland is large and irregular in form. It covers almost one-third of the posterior surface of the eye (figs. 15, 17, and 23, *Hg*), and extends from the region of the optic nerve forward as a broad lobular mass, narrowing abruptly to its duct where it passes under the proximal insertion of the superior and inferior oblique muscles. A large dorsal projection is

covered by the proximal portion of the rectus internus muscle, but the gland overlies the distal third of this muscle. The ventral portion of the gland partly conceals the inferior rectus and the inferior oblique muscles. MacLeod ('80) describes this gland in the duck as a typical tubular gland.

The blood supply to this gland is from the ophthalmotemporal branch of the external ophthalmic artery and from the ophthalmic vein (figs. 15 and 17). These, as they pass under the gland to supply the inferior oblique muscle, give off numerous branches to this gland which penetrate its under surface.

The nerve supply is from the inferior branch of the third nerve, which, as it passes under the gland to supply the inferior oblique muscle, gives off a twig to the under side of the gland which branches and ramifies over its under surface, fine branches penetrating the gland.

After the gland has narrowed to its duct at the anterior end, it lies under the two oblique muscles. The walls of the duct from this point become much thinner and lose their glandular appearance. The duct passes around the eye, closely adjacent to it, and opens into the anterior lower portion of the conjunctival sac under the nictitating membrane. The single opening is rather large and can be easily entered by passing a seeker under the nictitating membrane into this portion of the conjunctival sac (fig. 5, *H*). The very copious secretion is thus emptied near the region of the most active part of the nictitating membrane. At this point the conjunctival sac is more or less cup-shaped, which allows the accumulation of a considerable amount of fluid. The physiological significance of this is readily seen when we consider the movements of the nictitating membrane, previously described.

A better idea of the arrangement of the structures involved in the cleansing of the front of the eye can be had by consulting the diagram (fig. 7), a horizontal view. The large Harder's gland (*Hg*), situated back of the eyeball, pours out, by means of its duct (*Hd*), a copious secretion at the anterior canthus between the nictitating membrane and the eyeball. There is also a scanty secretion from the lacrimal gland (*Lg*) which enters at

the posterior canthus. The direction of movement of these secretions is indicated by the arrows. The structure of the nictitating membrane with its marginal plait is such that it not only favors the flow of tears through the lacrimal canal to the mouth, but, in my opinion, actually forces these secretions along.

Dissection of the lids from the posterior portion of the eye shows the two openings into the lacrimal canals (fig. 8, *ol*) at the

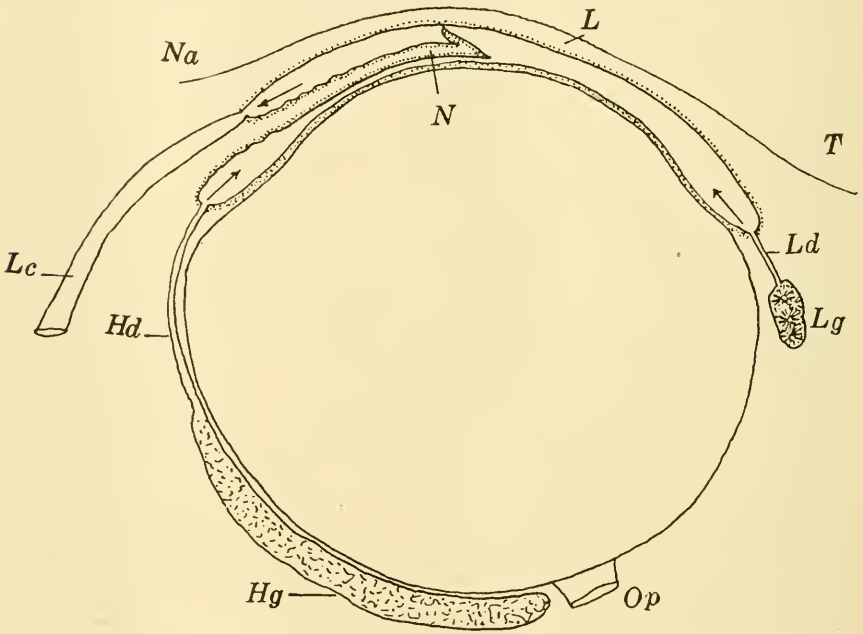


Fig. 7 Diagrammatic horizontal drawing of the relation of the glands and their ducts to the eye, the lids and the lacrimal canal leading to the roof of the mouth. *Hd*, duct of Harder's gland, *Hg*; *L*, eyelid; *Lc*, lacrimal canal; *Ld*, duct from the lacrimal gland, *Lg*;[9] *N*, nictitating membrane; *Na*, nasal, and *T*, temporal side of eye; *Op*, optic nerve. The arrows indicate the direction of flow of the lacrimal secretion.

anterior angle of the lids, close together and near the margin. That one the upper lid is larger than the lower. A groove—the peripalpebral groove—extends from each of these openings parallel to the margin of the lids. The upper groove is much deeper and longer, extending along almost the entire margin. It is deepest at the opening and gradually becomes less until it

disappears at the outer canthus of the eye. The function of these grooves is evidently to assist in directing the fluid to the openings of the canals and to prevent its escape over the margin of the lids.

The two lacrimal canals, which lead from the two openings on the under surface at the interior angle of the lids, are easily demonstrated by removing the skin covering them (fig. 9, *lc*). They lie just beneath the skin and extend in an anterior direc-

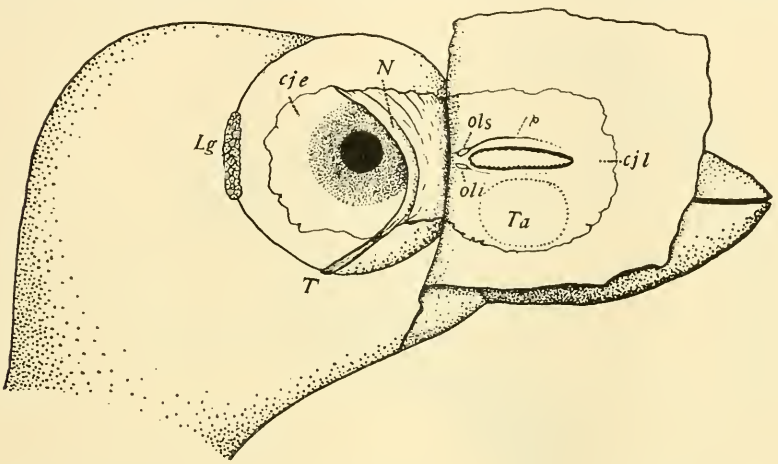


Fig. 8 Enlarged drawing of the sparrow head with the lids dissected and turned forward to show the openings of the lacrimal canals and the peripalpebral groove. *cje* conjunctiva of eye, and *cj l*, of lid; *Lg*, lacrimal gland; *N*, nictitating membrane; *oli* and *ols*, inferior and superior openings of lacrimal canals; *p*, peripalpebral groove; *Ta*, tarsus.

tion parallel to it. They widen near the openings into a more or less sac-shaped cavity. They are separated from each other for a distance of 2 or 3 mm. by a thin membrane; then they unite into the common duct near the base of the bill. This relatively wide duct now penetrates the bony structures and turns inward and downward to open at the lower portion of the nasal passage in the region of the choana (fig. 10, *L*). The secretions from the eye are thus directed almost into the mouth cavity, there being a distance of only about 2 mm. from the

opening of the duct to the roof of the mouth. In the normal position of the head the secretion would naturally drop this distance directly into the buccal cavity.

THE EXTRINSIC MUSCLES OF THE EYE

The muscles which move the eye of the bird in some respects closely resemble those of mammals. As in mammals, they include four rectus and two oblique muscles. Besides these, which function in moving the eyeball, birds possess also a pyra-

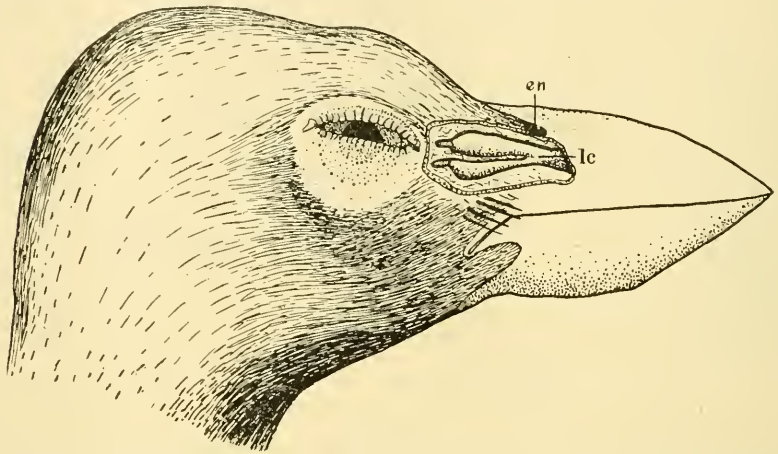


Fig. 9 Enlarged view showing the lacrimal canals exposed by the removal of skin covering them. *lc*, lacrimal canal; *en*, external nares.

midalis and a quadratus muscle whose contractions draw the nictitating membrane over the front of the eye.

In the sparrow the ribbon-like rectus muscles have their origin in the thick sheath surrounding the optic nerve, close to the optic chiasma. A marked difference is noticed in the relative length of these muscles in birds and mammals, due to the very short optic nerve and the relatively shallow eye socket in the bird. The main part of the muscular tissue of these muscles in birds is situated in the proximal two-thirds of their length. Each muscle widens distally, becomes thin, and finally loses its muscular tissue for the distal third of its course. Distally they

are inserted upon the eyeball a little beyond the equator by a broad, thin, and almost transparent tendons (figs. 11 and 23). The superior rectus (*Rs*) partially overlies the quadratus and the insertion of the superior oblique. The rectus internus (*R in*) is partly covered by Harder's gland (*Hg*) and the superior oblique (*Ob s*). The belly of the inferior rectus (*R inf*) is partly overlapped by Harder's gland. The insertion lies partly under that of the inferior oblique (*Ob i*). It conceals the pyramidalis.

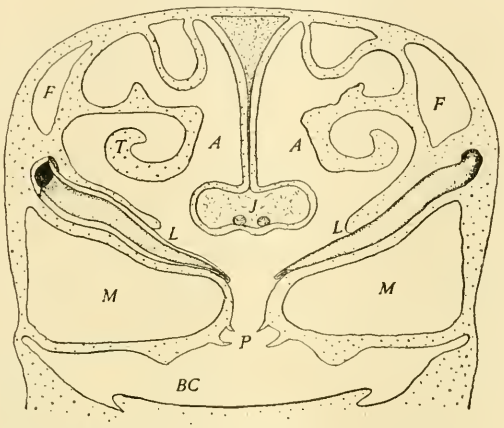


Fig. 10 Enlarged view of a cross-section of the head at the base of the beak about 2 mm. anterior to the eyes to show the lacrimal canals leading to the choana. *A-A*, right and left air passages connecting the external nares and the buccal cavity, *BC*, through the choana at *P; F*, frontal sinus; *J*, organ of Jacobson; *L*, lacrimal ducts opening by horizontal slit-like openings into the choana; the anterior wall of the right duct has been cut away; *M*, maxillary sinus; *T*, turbinals.

The insertion of the rectus externus (*R ex*) is partly concealed by the lacrimal gland.

The superior oblique (*Ob s*) and the inferior oblique (*Ob i*) have their origin at about the same place on the nasal side of the orbit, just above the duct from Harder's gland. The superior oblique extends directly from its origin to its insertion on the eyeball near that of the rectus superior. The pull is direct, and is not transmitted through a pulley-like arrangement as in mammals. The insertion of the inferior oblique is in the region of that of the rectus inferior which it partly overlies.

When the origins of the muscles are dissected loose and they are laid back (fig. 11) the quadratus (*Q*) and the pyramidalis (*P*) with its tendon (*T*) are easily seen. The shape of the quadratus resembles that of a thin section through a truncated pyra-

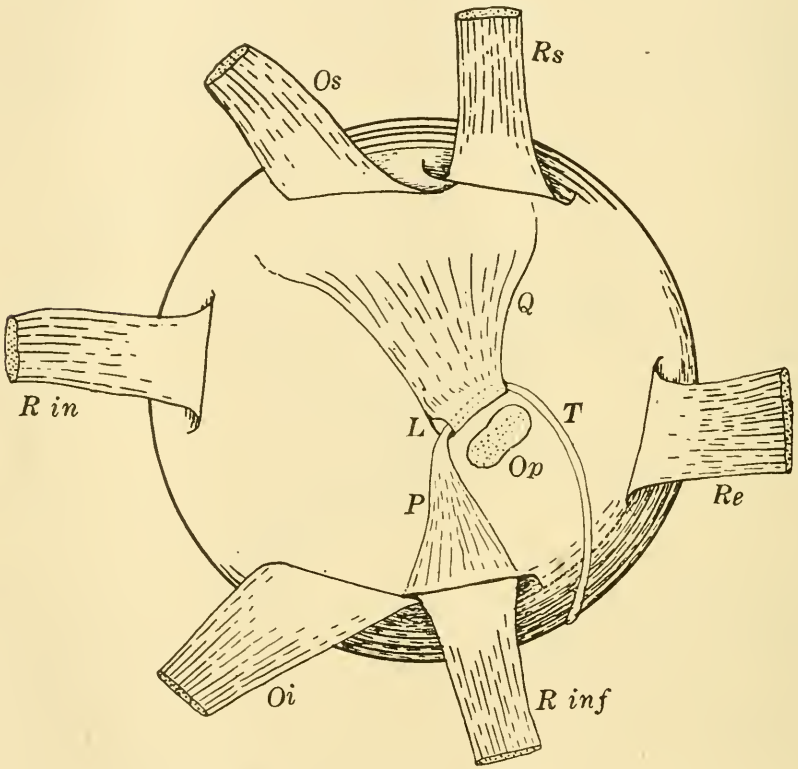


Fig. 11 Enlarged drawing of the posterior view of the right eye of the sparrow with the rectus superior (*Rs*), inferior (*R inf*), externus (*Re*), internus (*Rin*), and the superior (*Os*) and inferior (*Oi*) oblique muscles laid back to show the arrangement of the quadratus (*Q*) with its loop (*L*), and the pyramidalis (*P*) with its tendon (*T*), in relation to the optic nerve (*Op*).

mid. Its broad base is attached to the eyeball under the insertion of the superior oblique and superior rectus muscles. From this region it extends downwards and terminates abruptly in a broad, tendinous loop just above the optic nerve. The pyramidalis muscle, as its name implies, is much the shape of a thin

section through a pyramid. Its broad origin lies under the insertion of the inferior oblique and inferior rectus muscles. From this region it extends upward, lying close to the eyeball, and gradually narrowing until it terminates in a narrow flat tendon just anterior to the optic nerve. This tendon now turns backward, passes through the pully-like loop of the quadratus, just above the optic nerve entrance, and extends downward and backward, having almost completely encircled the optic nerve. After passing through the loop of the quadratus the pyramidal tendon lies in a groove, formed partly by the sclera and partly by the adjacent connective tissue. It is thus prevented from shifting its position, enabling it to pull always in the same direction. It thus extends around to the front side of the eye where it is attached to the lower movable part of the nictitating membrane. This insertion is mainly to the marginal plait of this membrane where it spreads out in a fan-like manner. Some strands of the tendon extend farther forward and are inserted into the lower part of the membrane. The united conraction of the pyramidalis and quadratus muscles is necessary to prevent the tendon of the pyramidalis from pulling down on the optic nerve and injuring it. This arrangement of muscles furnishes a greater amplitude of movement of the nictitating membrane than could be secured by the action of a single muscle. Both of these muscles are innervated by the same nerve.

By various experiments I have found that the sparrow can seldom be induced to close its true lids, even when the cornea is touched with an instrument. In fact, the true lids are apparently closed only in sleep or death and, in this bird, the nictitating membrane performs the function of the upper and lower lids of mamals.

Numerous observations show that the movements of the nictating membrane of each eye are more or less independent. They are often moved simultaneously, but one frequently moves across the eye while the other remains quiet.

It was further seen that the brightness of the light had a marked influence on the frequency of movement. When the sparrow was kept in the darker portion of the room, the num-

ber of movements was forty per minute, but when placed so that the eye was in direct sunlight the rate was almost doubled. When the nictitating membrane is stretched across the eye it is translucent, and the pupil can be readily seen through it. There is no doubt that the bird can perceive the presence of objects when this membrane is stretched over the eye. The importance of accomplishing, with a semitransparent membrane what is performed in other animals by opaque lids, can be readily appreciated when one considers the very rapid locomotion of the bird. When flying through woods or bushes—some birds being credited with a speed of as great as ninety miles an hour—the complete obstruction of vision by opaque lids for even a small fraction of a second would often result in a collision and death to the bird.

THE BLOOD SUPPLY TO THE EYE

The blood supply to the eye of the sparrow differs in many respects from that in man. Because of these differences and the small size of the eyeball and accessory parts in the sparrow the blood supply has been difficult to determine. It has been well nigh impossible to use the same names for the branches as are used for man because of the lack of conformity of the branches, but so far as possible, I have employed the names used in human anatomy. In order to get a clear idea of the blood supply to the eye it is necessary to describe the general arrangement of the arteries of the head.

The two common carotids unite into a single trunk a short distance from the heart, which, just before reaching the head divides into right and left common carotids. Figure 12 is a ventral view of the head and the main branches of the arteries. Each of these common carotids gives off a vertebral artery (*V*), and then divides into external (*CE*) and internal (*CI*) carotids. The three main branches of the external carotid (*Ppl*, *MI*, and *MS*) supply the palatine, inferior maxillary, and superior maxilary regions, respectively.

The internal carotid gives off a large branch, the external ophthalmic artery (*Op E*), which is directed outward to the

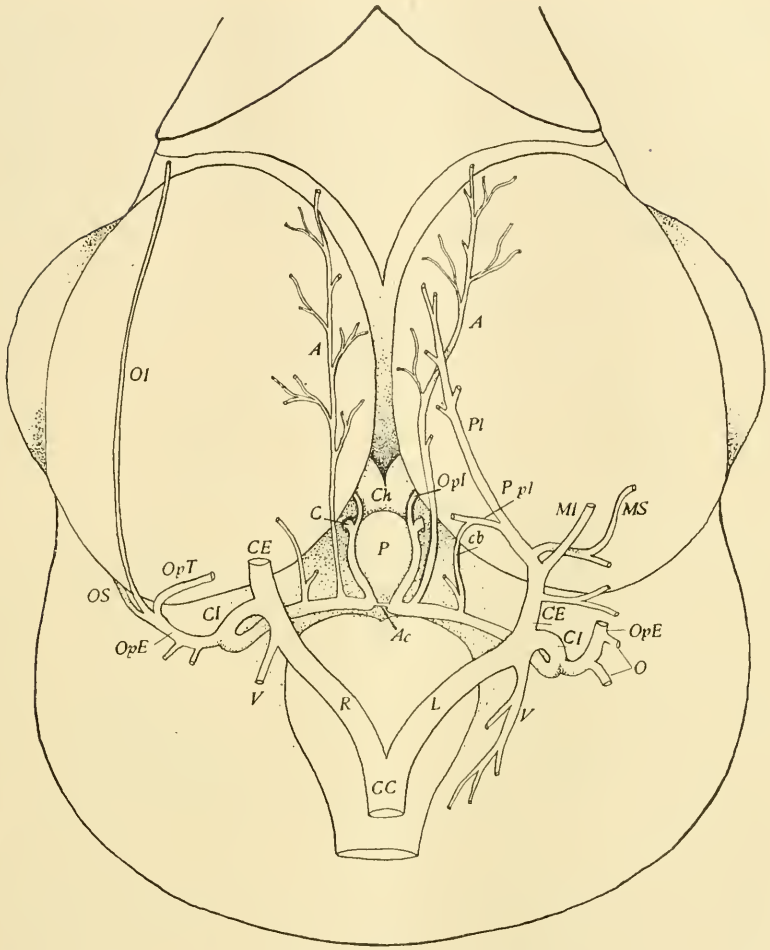


Fig. 12 Ventral view, showing the general arrangement of the arteries and the origin of those which supply the eye and its accessory parts. *A*, artery running forward just ventral to the capsule of Tenon to which it sends branches; *Ac*, artery (communicans) joining the internal carotids; *C*, cerebral artery passing dorsally to the brain; *cc*, common carotid; *Cb*, branch connecting the posterior palatine artery of the external carotid to the internal carotid; *Ch*, chiasma; *CE*, external carotid; *CI*, internal carotid; *L*, left common carotid; *MI*, inferior maxillary; *MS*, superior maxillary; *O*, occipital artery to the semicircular canals; *OI*, infra-orbital; *OS*, supra-orbital; *OpE*, external ophthalmic; *Op I*, internal ophthalmic; *OpT*, ophthalmotemporal; *P*, hypophysis; *Pl*, palatine; *Ppl*, posterior palatine; *R*, right common carotid; *V*, vertebral artery.

posterior portion of the eyeball. This branch gives off some occipital arteries (*O*) to the semicircular canals and to that portion of the head. When the external ophthalmic artery reaches the eye, it divides into three main branches; the superior ophthalmic (*OS*), the inferior ophthalmic (*OI*), and the ophthalmotemporal (*Gp T*). These branches will be described later.

After giving off the external ophthalmic artery the internal carotid turns inward and upward. For some distance it is enclosed in a tube of bone. It soon gives off a branch (*cb*) which connects the internal carotid to the palatine branch of the external carotid. A little farther on it gives off another branch (*A*) which is directed forward over the ventral surface of the orbit. For a short distance this vessel is also inclosed in a bony tube. In its course this vessel gives off branches to the tissues in this region and finally leaves the orbit at its anterior side. The internal carotid extends inward until it almost meets the internal carotid from the other side, just posterior to the hypophysis. Here the two carotids are united by a small commissural branch (*Ac*) which extends across in the groove between the floor of the brain and the hypophysis. At this point the carotid makes a sharp turn forward and, after encircling the hypophysis, divides into two branches: the cerebral (*C*), which runs dorsally to supply the brain; and the ophthalmic (*Opl*), which runs parallel with the optic nerve to supply portions of the eye.

As previously stated, the external ophthalmic artery divides into three branches; two of these, the superior orbital and the inferior orbital, supply the anterior portion of the eyeball, the conjunctiva, and the lids. Figure 13 shows the main distribution of these arteries as seen from the front.

The inferior orbital artery (*OI A*) runs forward along the ventral surface of the eye. In its course it gives off numerous branches which supply the skin and the lower portion of the lower lid. Some branches, after supplying the conjunctiva and structures in the inferior fornix, penetrate the sclerotic (*c*) posterior to its union with the cornea. These form the anterior ciliary arteries. The inferior orbital finally leaves the orbit at the anterior lower portion.

The superior orbital artery (*OS A*) runs up over the eyeball, partly encircling it, and finally leaves the orbit at the upper anterior portion. In its course it gives off a number of branches to the eye. Close to its origin from the external ophthalmic it gives off a small branch to the lacrimal gland. Another branch, the inferior palpebral artery (*PI*), goes to the lower lid. This supplies the main portion of the lower lid and forms a portion of

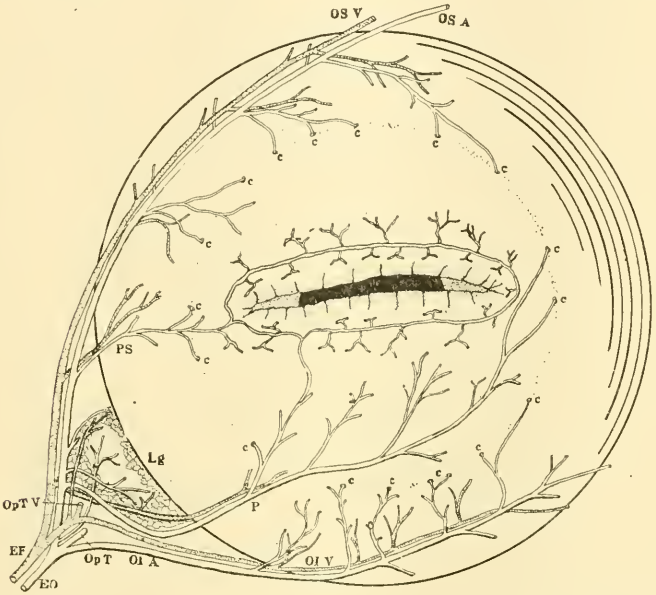


Fig. 13. Front view of the eye of the sparrow, the external layer of the lids being removed to show the arrangement of the arteries and veins. *c*, anterior ciliary arteries; *EF*, external facial vein; *EO*, external ophthalmic artery; *Lg* lacrimal gland; *OIA*, infra-orbital artery; *OSV*, supra-orbital vein; *P*, inferior palpebral artery and vein; *PS*, superior palpebral artery and vein. × 10.

a ring of blood-vessels which surround the interpalpebral space close to the margins of the lids. It also sends off a branch, the superior palpebral artery (*PS*), to the upper lid. It is united to the inferior palpebral by the small marginal artery which encircles the palpebral space. It forms also a few ciliary arteries. In its course over the eye the superior orbital gives off a number

of branches to the upper lid. to the tissues in the region of the superior fornix, and to the ciliary region. These ciliary arteries pierce the sclerotic in the region of the scleral plates (fig. 14).

The third branch of the external ophthalmic, the ophthalmotemporal, is directed backward, around the posterior side of the eye, to the region of the optic nerve. This artery is the main source of the blood supply to the extrinsic muscles, Harder's

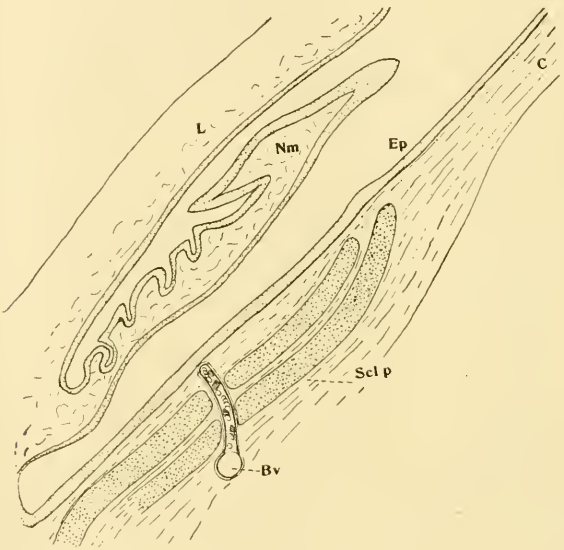


Fig. 14 Enlarged view of a section through the scleral plates (*Scl p*), showing the passage of a blood-vessel (*Bv*) directly through them. *C*, cornea; *Ep*; epithelium of cornea; *L*, lid; *Nm*, nictitating membrane.

gland, and the structures at the back of the eye. Figure 15 shows the distribution of the arteries over the posterior part of the eye. The general course of the ophthalmotemporal artery is inward and downward, passing under the external rectus, posterior to the optic nerve. It bends around under the nerve and ascends on the anterior side of it. Here it unites with one of its branches which has run forward over the dorsal side of the nerve and thus forms a complete ring around the optic

nerve. In its course it divides into a number of branches which will be described in more detail.

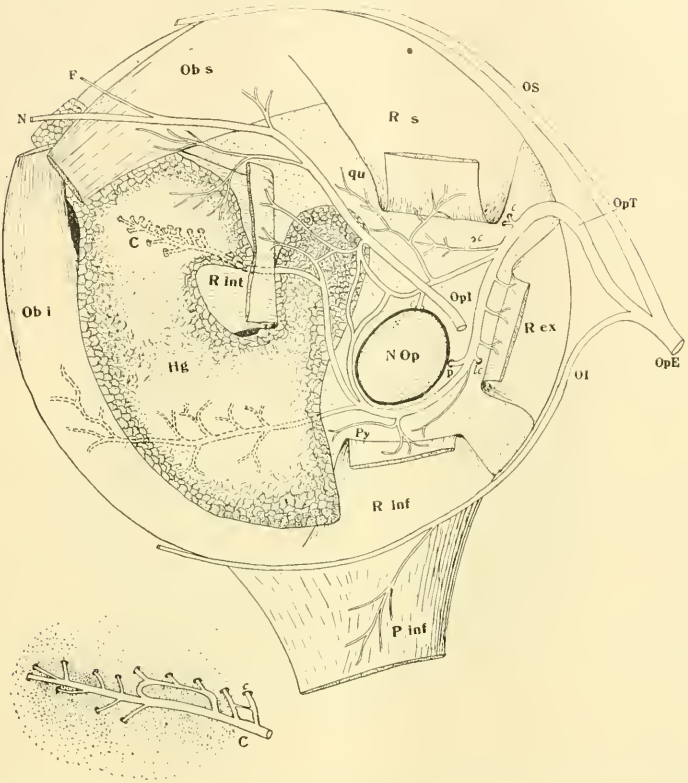


Fig. 15 Posterior view of the right eye of the sparrow, showing the arteries to the eye and its accessory parts. *C* and *c*, ciliary arteries; *F*, frontal branch of ophthalmic artery; *NOp*, optic nerve; *Ob i*, inferior oblique muscle; *Ob s*, superior oblique muscle; *Hg*, Harder's gland; *lc*, long ciliary artery; *N*, nasal branch of ophthalmic artery; *OI*, infra-orbital; *OS*, supra-orbital; *OpE*, external ophthalmic; *Op I*, internal ophthalmic; *Op T*, ophthalmotemporal branch; *p*, artery to pecten; *Pinf*, inferior palpebral muscle; *qu*, quadratus muscle; *Py*, pyramidalis; *R ex*, external rectus; *R inf*, inferior rectus; *R int*, internal rectus; *Rs*, superior rectus. × 10.

Soon after turning inward the ophthalmotemporal artery gives off some small branches which penetrate the sclerotic to supply the chorioid and ciliary muscles (*c*). It then sends off another

branch which, after supplying the superior rectus and the quadratus, gives off a small vessel which penetrates the sclerotic just above the optic nerve. After giving off this branch it unites with the main vessel which has almost completely encircled the optic nerve. It then extends forward and unites with a branch from the ophthalmic artery. These anastomoses are, no doubt, of great value in the blood supply to the eye. Owing to the very close fit of the eyeball in the orbit and the short optic nerve, the vessels on one side or the other would be compressed. by the movement of the eyes. In this case a compensatory flow of blood would occur through the vessels not compressed.

As the ophthalmotemporal artery passes under the external rectus, several branches are given off to the under or bulbar side of this muscle. Two other important branches are given off in this region; one to the pecten (*p*) and the other, the long ciliary (*lc*). The pectinal artery penetrates the sclera in the angle formed by the sclera and the sheath of the optic nerve. The long ciliary artery pierces the sclera slightly posterior to this.

On the ventral side of the optic nerve the ophthalmotemporal artery sends branches to the inferior rectus and pyramidalis muscles. A little farther on it divides into three branches, one of which passes under Harder's gland, supplying it with blood, and finally breaks up into small vessels in the inferior oblique. Another branch passes upward, and, after sending a branch to the internal rectus and one which anastamoses with the ophthalmic, proceeds under the muscle and gland to an oval pigmented region on the sclera. Here it divides into a number of branches which pierce the sclera as thirteen separate vessels (*C* and *Cc*). The third branch bends around the optic nerve, close to its anterior side, to unite with another of its branches and with the ophthalmic artery.

When the sclera is removed from the posterior part of the eye the distribution of the arteries which penetrate the outer tunic is seen. Figure 16 shows the larger vessels of the chorioid. At the point where the vessels stop in the figure they penetrate the chorioid and are so covered with pigment that they cannot readily be followed. They, however, spread out and anastomose

with each other to form a complete network in this great vascular coat. The finer divisions and branches are too intricate to show in such a figure.

The pectinal artery (*P*) divides as it enters the optic nerve tract. A better idea of the course of this artery can be had by consulting figures 73, 74, and 75, plate 12, which are photo-

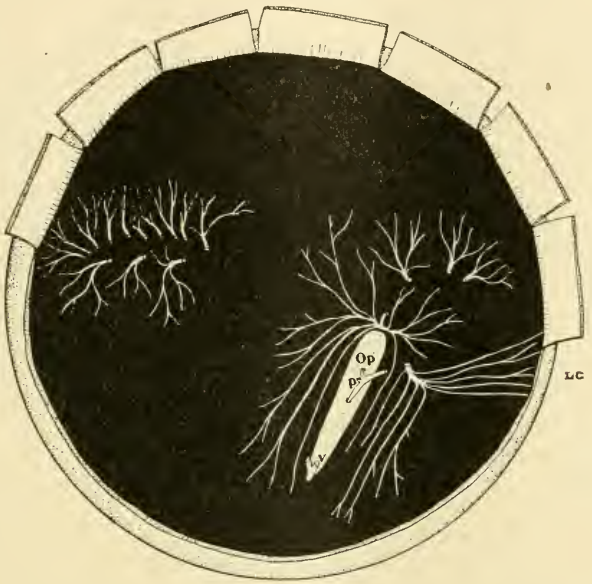


Fig. 16 Posterior view of the eye of the sparrow after the sclera has been removed to show the larger branches of the ciliary arteries and the artery and vein of the pecten. *Lc*, long ciliary arteries which run forward parallel with the long ciliary nerves; *Op*, optic nerve, showing its extension obliquely downward and forward; P, artery to pecten; *V*, vein from pecten. × 10.

graphs of sections at right angles to the pecten and vertical to the retina. In figure 75 a cross-section of the artery is shown, both in the angle of the optic nerve and eye on the outside, and almost at the base of the pecten. In figure 74 the course of the artery in its passage to the base of the pecten can be followed.

The veins carrying blood from the pecten emerge from the lower or distal end of the optic disc (fig. 16, *V*). A more detailed

description of the circulation of the pecten is given when dealing with this organ.

The other artery which supplies the eye is the ophthalmic (fig. 15, *Op I*). As already described, it arises from the internal carotid artery (fig. 12, *Op I*). After reaching the orbit it bends over the dorsal side of the optic nerve and passes in a fairly straight course to the anterior portion of the orbit. Here it divides and leaves the orbit as the facial (*F*) and nasal (*N*) arteries. In its course through the orbit it gives off a few branches, one of which anastomoses with the branches of the external ophthalmic and also supplies the internal rectus with a few vessels. A little farther on it sends branches to Harder's gland and the superior oblique muscle.

The arrangement of the veins of the eye is very similar to that of the arteries. The front of the eye is supplied by the external facial (fig. 13, *EF*). The names of the branches and their distribution are so like those of the arteries that it will be unnecessary to describe them. The distribution of the veins over the posterior part of the eye differs in some respects from that of the arteries (fig. 17). The ophthalmotemporal vein (*OpT*) divides into two large branches which pass the optic nerve on opposite sides. The anterior branch unites with the ophthalmic vein (*Op V*), the posterior branch receives two veins from the pecten, and then swings around under the nerve to unite with the ophthalmic near the union of the anterior branch. There is thus formed a venous circle around the optic nerve similar to that formed by the arteries.

The ophthalmic vein has a somewhat different course and different branches from the ophthalmic artery. It enters the orbit in the same region that the artery leaves it, and receives blood from the superior oblique, Harder's gland, the internal rectus and from the ophthalmotemporal vein. It passes anterior and ventral to the optic nerve and leaves the orbit at the lower side.

THE NERVE SUPPLY TO THE EYE

The nerve supply to the eye and its accessory parts is derived from five of the cranial nerves—the optic, oculomotor, trochlear, abducens, and trigeminus.

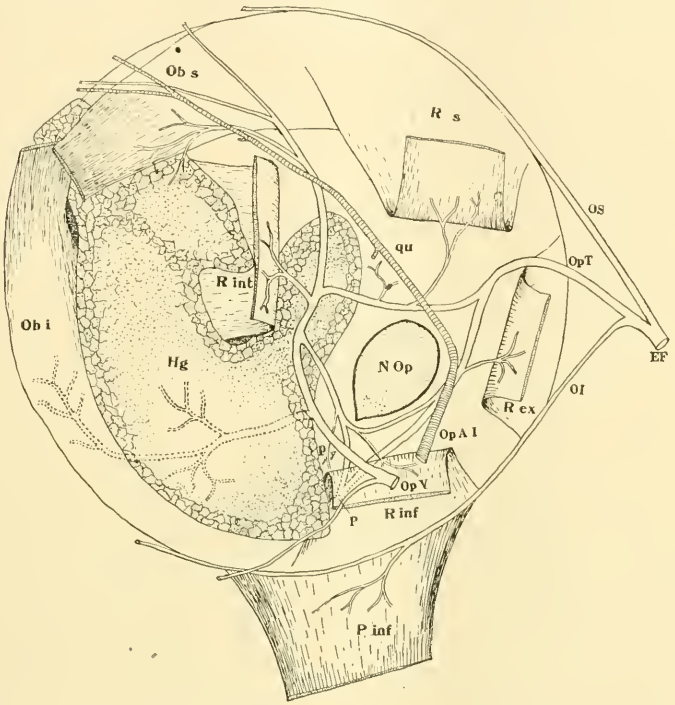


Fig. 17 Posterior view of the right eye of the sparrow, showing the venous supply to the eyeball and accessory structures. *EF*, external facial; *Hg*, Harderian gland; *N Op*, optic nerve; *Ob i*, inferior oblique; *Obs*, superior oblique; *OI*, infra-orbital; *OpAI*, internal ophthalmic artery; *OpT*, ophthalmotemporal vein; *Op V*, ophthalmic vein; *P*, point where vein from pecten pierces the sclera; *P inf*, infrapalpebral muscle; *Py*, pyramidalis muscle; *qu*, quadratus; *Rex*, external rectus; *R inf*, inferior rectus; *R inf*, internal rectus; *Rs*, superior rectus. × 10.

Figure 18, a ventral view of these nerves, shows their origin and some of their distribution. The optic nerve (*on*) is very short and thick. After leaving the chiasma (*ch*), it is directed outward and forward and runs straight to the eyeball. This

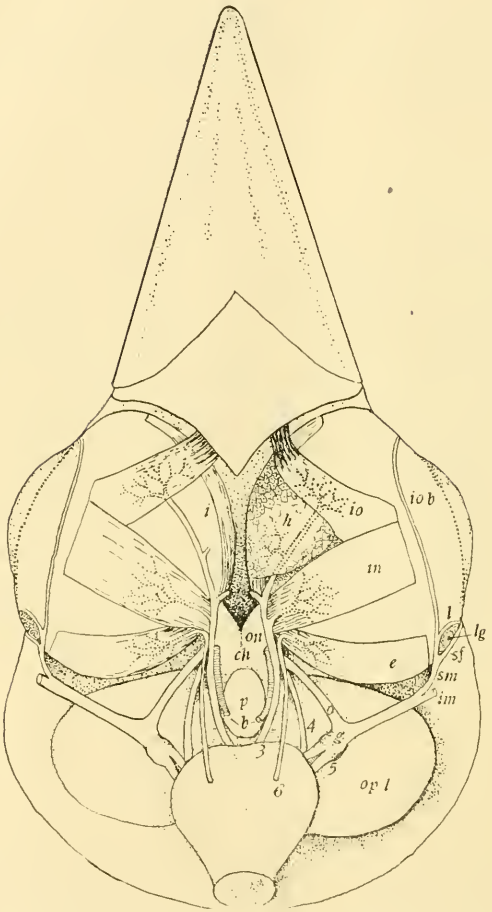


Fig. 18 Ventral view of the base of the brain, the eyes and the nerves to the eyeball and appendages. The Harderian gland of the right eye has been removed to show the internal rectus. *b*, blood-vessels; *ch*, chiasma; *e*, external rectus; *g*, Gasserian ganglion; *h*, Harderian gland; *i*, internal rectus; *im*, inferior maxillary nerve; *in*, inferior rectus; *io*, inferior oblique; *io b*, inferior orbital nerve; *lg*, lacrimal gland; *l*, lacrimal nerve; *o*, ophthalmic branch of the fifth nerve; *on*, optic nerve; *op l*, optic lobe; *p*, hypophysis; *sf*, superior orbital or frontal nerve; *sm*, superior maxillary; *3*, *4*, *5*, and *6*, third, fourth, fifth, and sixth cranial nerves. × 4.

short, thick stump is very different from the optic nerve in man. The optic tracts lead directly from the chiasma to the large optic lobes (*op 2*). There is a complete decussation of the optic fibers at the chiasma. The fibers cross not as a network of individual fibers, but in flat ribbon-like bundles which alternate with each other. There are eight or nine of these bundles from each side. This has been previously described by Meckel

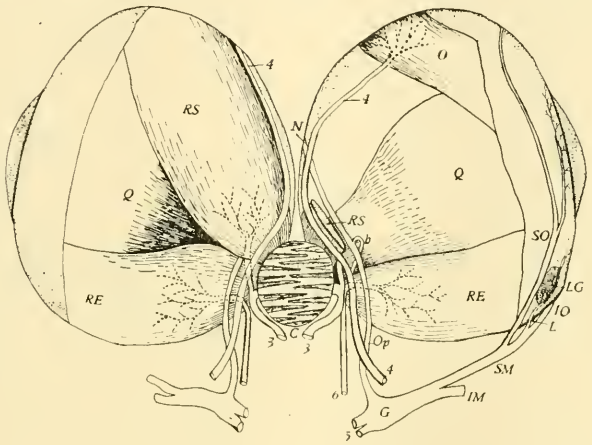


Fig. 19 Posterior view of the eyes of the sparrow after the brain, the skull and lower mandible have been removed, to show the relations of the different parts. The superior rectus of the right eye has been removed to show the nerves and muscles lying beneath. *C*, chiasma; *b*, branch from ophthalmic to third nerve; *G*, Gasserian ganglion; *IM*, inferior maxillary; *IO*, inferior orbital; *L*, lacrimal nerve; *LG*, lacrimal gland; *N*, nasal nerve; *O*, superior oblique; *Op*, ophthalmic nerve; *Q*, quadratus; *RE*, rectus externus; *RS*, rectus superior; *SM*, superior maxillary; *SO*, superior orbital; *3*, *4*, *5*, and *6*, the third, fourth, fifth and sixth cranial nerves. × 5.

(1816), Biesiadecki ('61), and Michel ('73) as characteristic of birds. They further claim that the number of these leaf-like bundles varies with different species of birds. Meckel found from fourteen to fifteen such bundles from either side in the crow. Figure 19 is a posterior view of the eyes and the nerves. The chiasma is cut across just anterior to the hypophysis and shows the ribbon-like bundles of fibers. Figure 20 shows the appearance of the eye structures as seen in a vertical section through

the median plane of the head. These figures show also the position and distribution of the nerves as they appear in these dissections. Figures 31 and 32, plate 6, are horizontal sections of the head through the chiasma. The crossing of the fibers in ribbon-like bands can be seen.

The optic nerve enters the eye some distance toward the outer and lower side from the axis of the eye. Its fibers are directed downward and forward. After piercing the seclera, the main

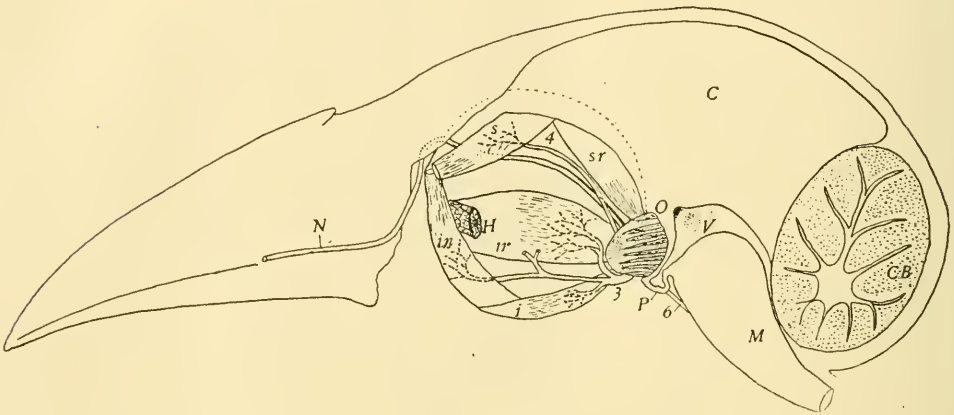
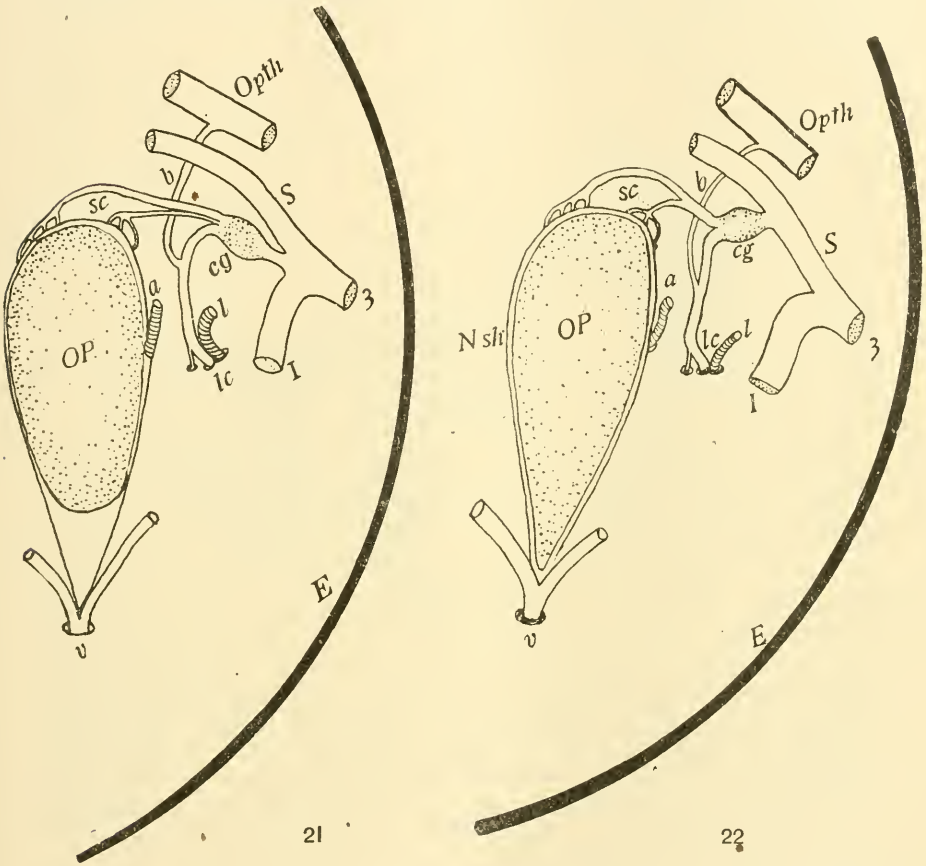


Fig. 20 Median section through the sparrow head, showing relations of the different parts. The lower mandible and the ventral portion of the skull have been removed. *C*, cerebrum; *CB*, cerebellum; *H*, Harder's gland; *i*, inferior rectus; *in*, inferior oblique; *ir*, internal rectus; *M*, medulla; *N*, nasal nerve; *O*, optic nerve; *P*, hypophysis; *s*, superior oblique; *sr*, superior rectus; *V*, third ventricle; *3*, *4*, and *6*, third, fourth, and sixth cranial nerves. × 4.

bulk of its fibers extend downward and forward, becoming less and less numerous as they proceed, until they finally disappear over the surface of the retina almost at the ora serrata. The shape of the optic disc thus formed is seen in figure 22.

The third or oculomotor nerve arises from the anterior edge of the pons (fig. 18) and, running parallel with the cerebral artery (*b*), bends out around the hypophysis. On reaching the region of the optic nerve it divides into two main branches, the superior of which runs dorsally, posterior to the optic nerve, to supply the superior rectus muscle (fig. 23, *S*). A very small

branch of this superior division appears to reach the superior palpebral muscle. Another small branch is sometimes given off to the ciliary ganglion (fig. 22). The origin of this small branch is variable (fig. 24, *C*, and figs. 21 and 22, *cg*). This



Figs. 21 and 22 Posterior view of a portion of the right eyeball after removal of the muscles, showing the ciliary ganglion and its relations to the various nerves, to show variations in their branching. *a*, artery which pierces the sclera at the angle of the sheath of the optic nerve and to supply the pecten; *b*, branch from the ophthalmic nerve; *cg*, ciliary ganglion; *E*, margin of eyeball; *I*, inferior branch of third nerve; *lc*, long ciliary nerve; *N sh*, sheath of optic nerve; *OP*, optic nerve; *Opth*, ophthalmic branch of fifth nerve; *S*, superior branch of third nerve; *v*, point of emergence through the sclera of the vein from the pecten; *3*, third nerve. × 20.

branch and the ciliary ganglion will be described later. The other branch, the inferior, extends forward over the ventral surface of the optic nerve and sends branches to the inferior rectus, inferior palpebral, internal rectus, inferior oblique and Harder's gland. Four of the muscles which move the eyeball are thus innervated by this nerve.

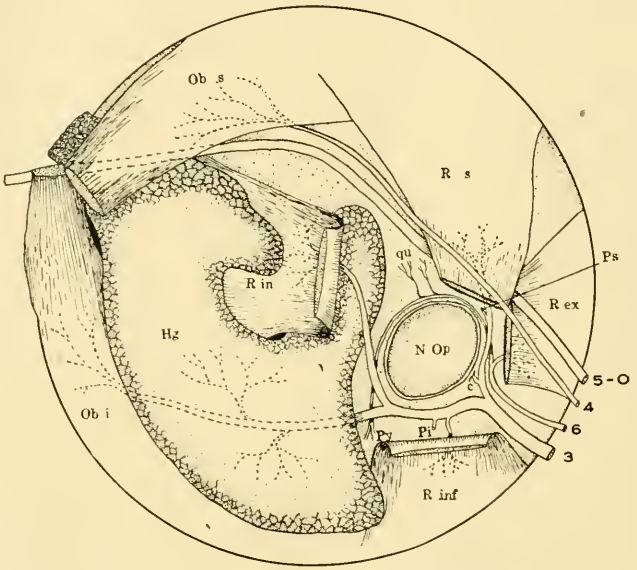


Fig. 23 Posterior view of the sparrow eye, showing the normal relative position of nerves, muscles, and glands. *R ex*, external rectus; *H*, Harder's gland; *ob i*, inferior oblique; *in r*, internal rectus; *R inf*, inferior rectus; *n*, optic nerve; *qu*, quadratus; *os*, sheath of optic nerve; *ob s*, superior oblique; *rs*, superior rectus; *3*, third nerve with its superior and inferior branches; *4*, fourth nerve; *5–O*, ophthalmic branch of fifth nerve; *6*, sixth nerve; *Py*, pyramidalis; *qu*, quadratus; *Ps*, posterior ciliary nerve; *c*, ciliary ganglion; *Pi*, inferior palpebral branch of third nerve.

The fourth nerve, the trochlearis, arises from the dorsolateral side of the medulla. It bends around the medulla to the ventral surface where it emerges at the anterior margin of the pons in the groove formed by the pons and the optic lobe (fig. 18, *4*). From this point it runs almost straight forward. It passes over the dorsal side of the optic nerve directly to the superior oblique

which it innervates (fig. 23, *4*). In its course it lies close to the orbital wall, outside of the external and superior rectus muscles, until near its termination, when it dips under the superior oblique and branches over its bulbar surface.

The sixth nerve, the abducens, arises from the ventral surface of the medulla near the median line (fig. 18, *6*). It runs forward almost parallel with the trochlear nerve to the posterior side of the optic nerve. Here it divides into two parts (fig. 23, *6*). The larger division turns sharply outward and passes under the external rectus where it divides into fine branches which penetrate the bulbar side of this muscle. The smaller division passes under the rectus muscles close to the eyeball. Its course is upward over the dorsal side of the optic nerve and down the anterior side where it finally terminates in the pyramidalis muscle. It thus almost encircles the optic nerve. In the region of the quadratus it gives off branches which innervate this muscle.

The fifth or trigeminus nerve arises at the side of the anterior portion of the medulla by two roots (fig. 18, *5*). These unite at the Gasserian ganglion (*g*) a short distance from the medulla. At the distal margin of the Gasserian ganglion the ophthalmic nerve (*o*) is given off. This runs forward and enters the orbit posterior to the optic nerve. Its course is in a dorsal and anterior direction around the eye (fig. 23, *5*). It leaves the orbit at the anterior margin near the region of the duct from Harder's gland. In its course through the orbit it runs almost parallel with the trochlearis. Just after entering the orbit it lies between the external rectus and the orbital wall. It then passes under the superior rectus, just dorsal to the optic nerve, over the quadratus and under the superior oblique to its exit from the orbit. In the region of the optic nerve a small branch is given off, which runs under the muscles to the eyeball where it turns downward and joins a branch from the ciliary ganglion (fig. 24, *bo*, figs. 21 and 22, *b*).

The long and short ciliary nerves in the sparrow are derived from the oculomotor and the ophthalmic nerves, as already stated. A more detailed description will, however, be necessary, as some variations have been found.

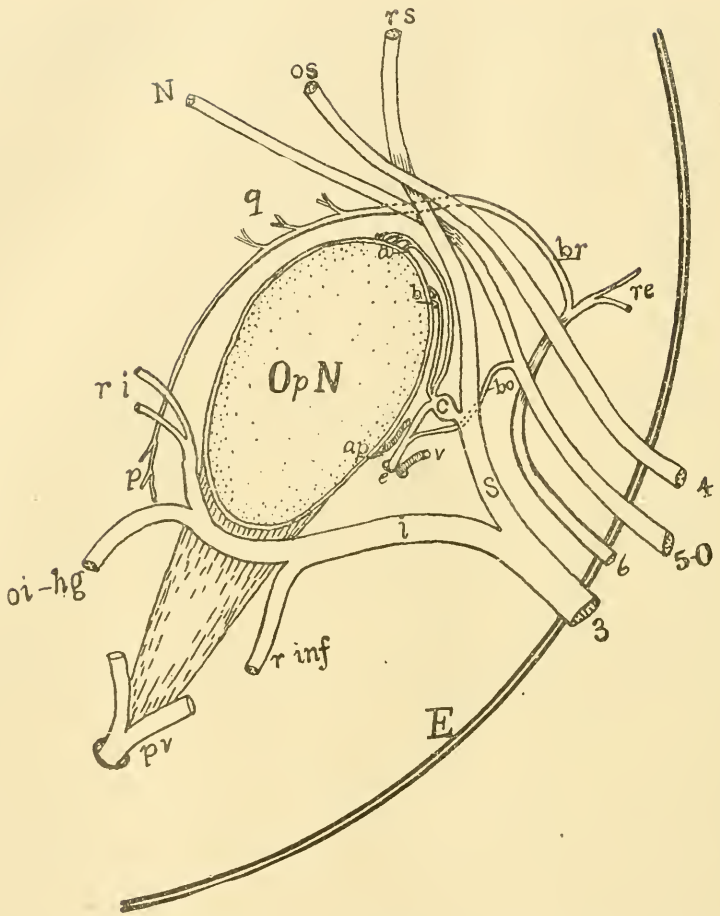


Fig. 24 Enlarged view, showing relative positions of the nerves of the eye in the sparrow. *a*, point where superior branch from ciliary ganglion penetrates the sclera; *ap*, artery to pecten penetrates sclera; *b*, point where median branch from ciliary ganglion penetrates the sclera; *br*, branch from sixth nerve to quadratus (*q*) and pyramidalis (*P*); *c*, ciliary ganglion; *bo*, branch from the ophthalmic to inferior branch of third nerve; *E*, margin of eyeball; *e*, where inferior branches from ciliary ganglion enters the sclera together with a small artery (*v*); *i*, inferior branch of third nerve; *N*, nasalis; *p*, to pyramidalis; *pv*, vein from pecten; *q*, to quadratus; *Op N*, optic nerve; *os*, to superior oblique; *oi*, *hg*, to inferior oblique and Harder's gland; *re*, to external rectus; *ri*, to internal rectus; *r inf*, to inferior rectus; *rs*, to superior rectus; *3*, third nerve; *4*, fourth nerve; *5–O*, ophthalmic branch of fifth; *6*, sixth nerve.

The ciliary ganglion (*cg*, figs. 21, 22, and 24, *c*) is situated directly on a branch of the third nerve. This branch may be given off at the point of division of this nerve into the superior and inferior branches as seen in figure 21, or it may leave the superior branch of the third as far from the main division as shown in figure 22. The branch connecting the ganglion with the third nerve is very short. In fact, the position of the ganglion could almost be described as located on the side of the nerve. From the distal side of the ganglion I have found in all cases at least two branches and sometimes three. When but two branches occur, one of these divides very soon into a small and a large branch, varying in the location at which the small branch is given off. These two nerves run to the dorsal side of the optic nerve. The smaller branch divides into three parts which immediately pierce the sclera in three places in the angle formed by the optic sheath and the sclera. The larger branch extends a little farther forward. It divides into four fine branches which pierce the sclera at four distinct points in this same angle (*sc*, figs. 21 and 22). These little branches form seven of the short ciliary nerves.

The other large branch which leaves the ciliary ganglion is directed downward along the posterior side of the optic nerve. It is joined about midway of its course by the small branch from the ophthalmic nerve. This common trunk extends on downward to where the long ciliary artery pierces the sclera. Here it divides into a large and a small branch which immediately penetrates the sclera at two places.

When the sclera is removed, the distribution of these nerves over the chorioid is readily seen (figs. 25 and 26). The seven short ciliary nerves, referred to above, radiate in practically all directions from the place of entrance. The distribution of the nerve formed by the union of the branches from the third and fifth nerves is as follows: The smaller branch, visible on the outside of the eye, is a short ciliary nerve. The larger branch divides at once after reaching the chorioid into the two long ciliary nerves which lie on the surface of the chorioid and extend around the lateral side to the ciliary region (fig. 26). Here they

divide into numerous branches which form a nerve plexus (*Cp*) extending completely around the eye.

The arrangement of the ciliary nerves in relation to the ciliary ganglion of the sparrow, as I have found it, differs in some respects from that in the hen as described by Carpenter ('11) and Lenhossek ('11).

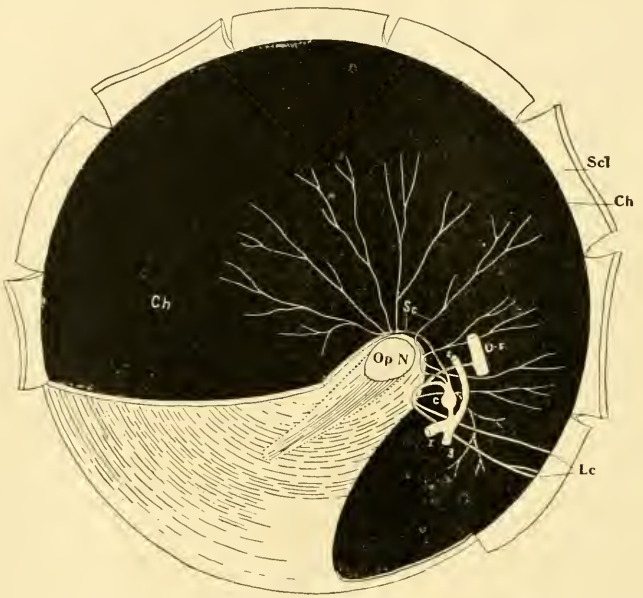


Fig. 25 Drawing of the posterior view of the right eye from a dissection, showing the distribution of the long and short ciliary nerves. *C*, ciliary ganglion; *Ch*, chorioid; *I*, inferior branch of third nerve; *Lc*, long ciliary nerves; *Op N*, optic nerve; *O-5*, ophthalmic nerve; *S*, superior branch of third nerve; *Sc*, short ciliary nerves; *Scl*, sclera; *3*, third nerve.

Carpenter finds one large branch and a variable number of smaller branches leaving the distal side of the ciliary ganglion. He also found that the branch from the ophthalmic nerve divides into two. One of these unites with one of the nerves from the ciliary ganglion; the other runs directly to the eye and forms the long ciliary nerves. He describes the ganglion as situated directly on the third nerve and not connected by a short branch He claims that all the branches from the distal portion of the

ganglion form short ciliary nerves. The communicating branch from the ophthalmic which unites with one of these distal branches sends a few fibers into the ganglion. The remaining fibers run along with the distal branch to the eye and form long ciliary nerves. He finds that the cells of the ganglion are unipolar and that their unbranched processes form the short ciliary

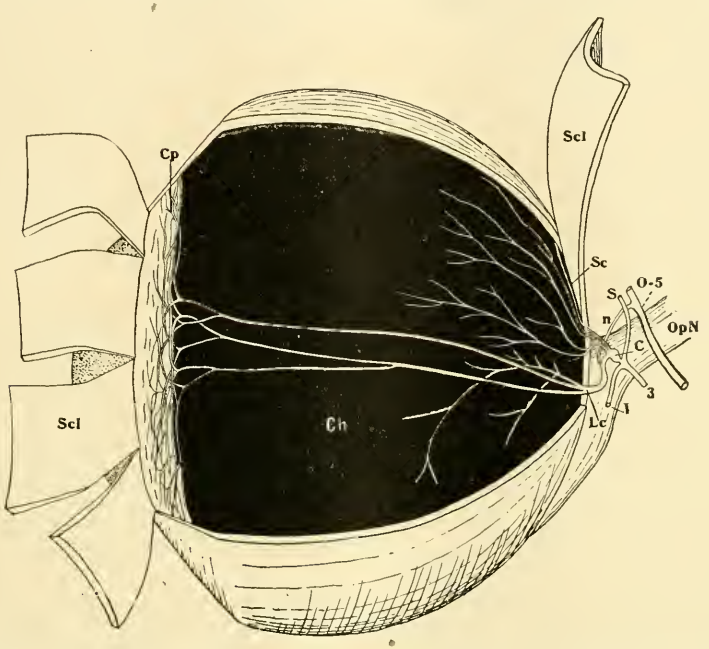


Fig. 26 Drawing of the lateral view of the left eye from a dissection, showing the distribution of the long and short ciliary nerves. *C*, ciliary ganglion; *Ch*, choroid; *Cp*, ciliary plexus in ciliary region; *I*, inferior branch of third nerve; *Lc*, long ciliary nerves; *n*, branch from ophthalmic nerve connecting with third; *Op N*, optic nerve; *0-5*, ophthalmic nerve; *S*, superior branch of third nerve; *Sc*, short ciliary nerves; *Scl*, sclera; *3*, third nerve.

nerves. Fibers from the third nerve enter the ganglion and terminate in brush-like endings about the cells in the proximal three-fourths of the ganglion. The fibers from the ophthalmic which enter the distal portion of the ganglion terminate about the remaining cells in the distal fourth. He failed to find a sympathetic root. Stimulation of the oculomotor nerve causes

constriction of the pupil. Stimulation of the ophthalmic nerve causes dilation of the pupil. His final conclusion is: "The ciliary ganglion of birds is not cerebrospinal, nor, strictly speaking, sympathetic. It appears to be a purely motor ganglion, with peculiar histological characters, belonging to the midbrain and bulbar subdivisions of the autonomic nervous system."

Lenhossek found the ciliary ganglion joined to the third nerve by a short branch. He describes two branches of unequal size leaving the distal side of the ganglion. The smaller branch runs directly to the eyeball and the larger is joined by a branch from the ophthalmic nerve. He also describes the branch from the ophthalmic as dividing into two parts. One of these divisions goes directly to the eyeball and forms the long ciliary nerves. The other division joins the large branch from the ciliary ganglion.

In my dissection of the sparrow I have not been able to find such a division in this ophthalmic branch; but, owing to the very small size of this branch, I may have overlooked it. It appears to run undivided to join one of the branches from the distal side of the ciliary ganglion. Also, the nerve formed by the union of the nerve from the ciliary ganglion and the ophthalmic branch gives rise to the long ciliary nerves and one short ciliary nerve. The arrangement in the hen is therefore quite different from what I find in the sparrow.

The fifth nerve, or trigeminus, after giving off the ophthalmic runs outward and forward. It soon divides into two branches of unequal size. The larger and ventral division, the inferior maxilary, runs directly to the lower mandible. The smaller or dorsal branch, the superior maxillary, extends upward and outward to the region of the lacrimal gland (fig. 27), where it divides into two branches, a superior (supra-orbital or frontal, *so*) and an inferior branch (infra-orbital, *io*).

The supra-orbital nerve runs upward over the eye at the edge of the orbit just beneath the skin to which it gives numerous branches. On the dorsal side of the eye it divides into two branches, one passing into the orbit and the other running forward to leave the orbit at the upper anterior portion.

The infra-orbital branch of the superior maxillary nerve, after sending off some small branches to the lacrimal gland, divides into two parts. The superior division, the lacrimal nerve, (*l*), is directed upward and forward along the anterior and lower margin of the lacrimal gland just beneath the skin. It sends some small nerves to the gland and then divides near the posterior canthus into two branches. The superior supplies the upper lid; the inferior, after sending branches to the lower lid,

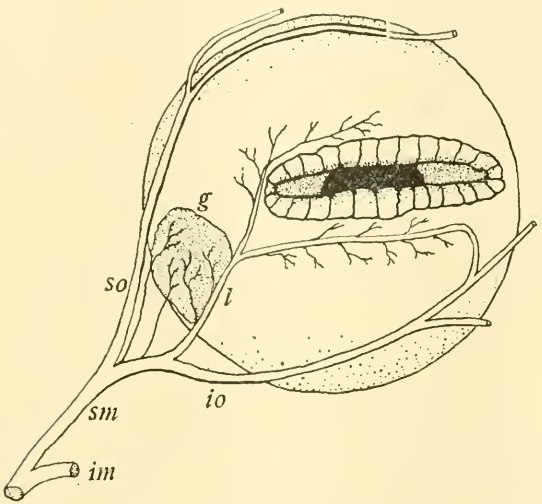


Fig. 27 Enlarged view of the right eye of sparrow, showing the distribution of the nerves over the front of the eye, the skin, with the exception of the margin of the lids, having been removed. *g*, lacrimal gland; *im*, inferior maxillary nerve; *io*, inferior orbital nerve; *l*, lacrimal nerve; *sm*, superior maxillary nerve; *so*, superior orbital nerve.

turns downward and joins the infra-orbital near the anterior portion of the eye.

The infra-orbital, after giving off the lacrimal branch, runs forward over the ventral surface of the eye near the margin of the orbit, near the anterior part of which it divides. The upper branch is joined by a part of the lacrimal nerve as stated above. These two divisions of the infra-orbital soon leave the orbit and are distributed over the skin and upper mandible.

The distribution of the nerves to the orbit may be briefly summarized as follows:

The oculomotor nerve supplies the superior rectus, the inferior rectus, the internal rectus, the inferior oblique, and the palpebral muscles, and Harder's gland. Through the ciliary ganglion and short ciliary nerves it supplies the chorioid and possibly some of the long ciliary nerves to the ciliary muscles and the muscles of the iris. It gives rise to at least seven of the short ciliary nerves.

The trochlearis goes to the superior oblique muscle.

The abducens supplies the external rectus, the quadratus, and the pyramidalis muscles.

The ophthalmic branch of the trigeminus sends a branch which unites with a nerve from the ciliary ganglion from which are formed the two long ciliary nerves and one short ciliary nerve.

The superior maxillary branch of the trigeminus innervates the lacrimal gland, the conjunctiva, the lids, and adjacent parts.

THE LENS

The lens of the sparrow differs in many respects from that of man. It is composed of a firm central part, almost spherical in shape, and a less firm peripheral portion (fig. 28, *Lns*, plate 5). The central portion forms the main bulk of the lens and is the part which functions in sight.

This central part is completely surrounded by a ring-like pad which forms the periphery of the equator of the lens adjacent to the ciliary processes. This structure is described by Rabl ('98) and Ritter ('00) as the 'Ringwulst.' I have called it the annular pad (fig. 28, *Ap*, plate 5). In a preserved lens it is easily separated from the hard, central, spherical part. In fact, in making sections through the whole eye, it is often difficult to keep the firm center from dropping out of the section. When these two parts of the lens are separated, the center resembles a hard, bullet-like mass and the annular pad a wedding ring.

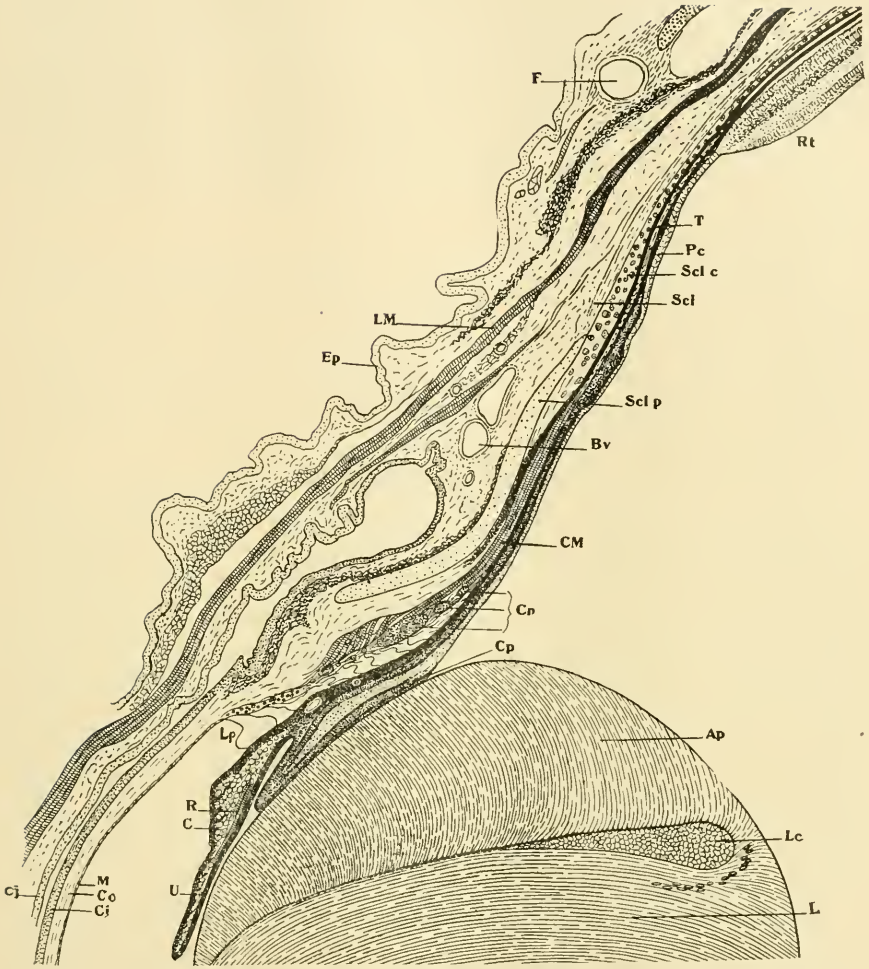


Fig. 28 Enlarged drawing of a vertical section of the lower lid and the ciliary region, showing the relation of the different parts. *C*, circular muscles of the iris; *Bv*, blood-vessels; *Cj*, conjunctiva; *CM*, ciliary muscles; *Cn*, ciliary nerves; *Co*, stratified portion of the cornea; *Cp*, ciliary processes; *Ep*, epithelium; *F*, feather follicle; *L*, lenticular portion of the lens, *Lc*, lenticular chamber, *LM*, depressor palpebral muscle; *M*, membrane of Descemet; *Lp*, ligamentum pectinatum; *Pc*, pars ciliaris retinae; *R*, radial muscles of the iris; *Rt*, retina; *Scl*, sclera; *Scl c*, scleral cartilage; *Scl p*, scleral plates; *T*, posterior tendon of the ciliary muscles attached to the sclera posterior to the ora serrata; *U*, uvea of iris.

The annular pad is separated from the lens proper by a space of variable thickness (fig. 28, *Lc*), the lenticular chamber, which is filled with lymph and granules arranged in clusters of indefinite shape. The lenticular chamber, as seen in a section along the axis of vision, resembles somewhat a comma in shape. Its larger, globular part is near the posterior margin of the annular pad. It narrows as it extends forward until it disappears at the anterior margin of the pad.

According to Henle ('79) and Ritter ('00) the lenticular chamber plays an important part in accommodation in the bird. I think its function is mainly if not wholly that of nourishment. Development shows it to be the remains of the embryonic lens cavity. Its location is at the line of junction of the inner surface of the lens capsule.

The corneal part of the annular pad is not very sharply separated from the lens proper. The cells at the anterior margin are short and radially arranged with the center of the lens as a centre. The single row of nuclei is situated near the peripheral ends of the cells. Toward the equator of the lens these cells become gradually longer, six-sided prisms. Instead of radiating directly from the center of the lens, they are more and more bent, with the concave side forward (fig. 28, *Ap*). The greatest curvature of these cells occurs at the thickest portion of the annular pad or the equator of the lens. Toward the posterior margin of this pad these cells become less and less bent, until at the extreme posterior margin they are almost straight and again radiate as before.

The inner ends of the cells of the annular pad appear to be globular. This is more pronounced from the equatorial region to near the posterior margin or where the lenticular space is widest. When these cells are examined with an oil-immersion lens one is impressed by their glandular appearance. The material filling the lenticular chamber also appears to be a coagulated secretion. The globular portion, which under low power looks like the inner ends of the cells, is seen to be a granular mass protruding from the end of the cell. The granules are not distributed uniformally throughout this mass, but there is a space

adjacent to the end of the cell which is almost free from granules. From this region there is a gradual increase in the number of granules toward the free margin of the globular mass. This granular substance can be traced into the cells, in many cases practically to their peripheral ends (fig. 29). These globular

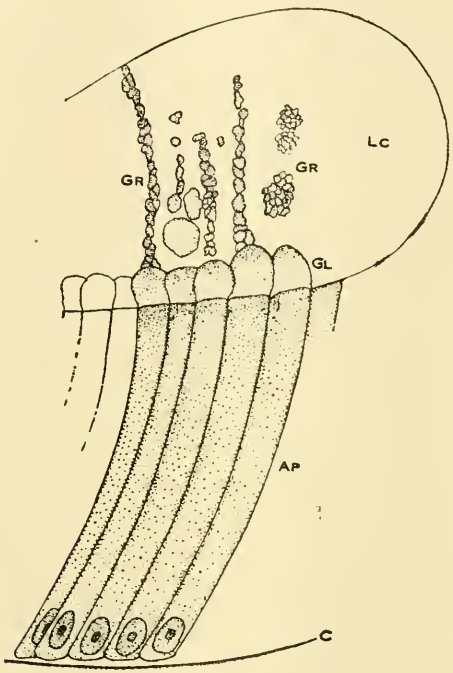


Fig. 29 Enlarged view of the cells of the annular pad, showing the secretions at the inner ends and the granular contents of the lenticular chamber. The cells are full of granules similar to those found in the lenticular chamber. *AP*, cells of the annular pad; *C*, capsule of the lens; *Gl*, secretion from cells coagulated in globular form; *GR*, irregular masses of granules into which the globular masses break up; *LC*, lenticular chamber.

masses occur free in the lenticular chamber in all stages of breaking up into individual granules. They may be as perfect as the mass at the end of the cell or may be broken up into many smaller, irregular aggregates, the most perfect globules being found nearest the ends of the cells. The fact that these

globular masses are found free in the lenticular chamber as well as attached to the cells leads me to infer that they are secretions from the cells of the annular pad and that they have assumed globular and irregular forms due to coagulation by the hardening fluids. The granular appearance of the cells also indicates that they are secretory in function. Rows of granular masses can often be seen extending directly away from the bases of the cells, frequently reaching the opposite side of the lenticular chamber. They are apparently formed by the rapid secretion of the cells to which they are adjacent. The contents of the lenticular chamber is, in my opinion, a secretion formed by the cells of the annular pad. This secretion serves to nourish the lens. The globular ends of the cells described by many authors is simply this coagulated secretion and lies wholly outside the cell wall. The function of the annular pad and the lenticular chamber would therefore be nutritive. This structure does not indicate that they could take any part in accommodation as stated by Ritter ('00). Any strain or pressure on the annular pad could not be transmitted to the lenticular part because of the fluid content of the lenticular chamber.

The nuclei of the cells of the anterior and posterior margins of the annular pad are located at the extreme outer ends close to the lens capsule and are very conspicuous. In the region of the equator they form two rows and are located a short distance from the peripheral ends of the cells. At either margin only one row is found, which gradually disappears. This leaves the anterior and posterior surfaces and the entire central portion of the lens free from nuclei which can be demonstrated by haematoxylin and eosin stains.

The cells of the anterior and posterior margins of the annular pad differ. Those in front are quite similar in structure to those of the equator. Toward the posterior margin the cells gradually lose their granular appearance their nuclei are no longer visible and they show a gradual transformation into the true lens fibers. A sharp line can therefore not be drawn separating the cells of the posterior margin of the annular pad from those of the central mass or true lens.

THE SCLERA

The sclera of the bird eye differs in shape, thickness, and structure from that of man. The curvature differs in various portions (fig. 30). The posterior part is very symmetrical and forms practically a segment of a hollow sphere whose center is at the middle of the posterior surface of the lens. This segment extends forward to near the region of the ora serrata where it turns rather abruptly toward the lens. Near the equatorial margin of the lens it unites with the corneal part of the sclera.

The corneal part represents a segment of a much smaller sphere, the ratio of which to that of the larger is 1: $2\frac{1}{4}$. The diameter of the base of the corneal segment, or at the junction of the cornea and sclera is 1.8 mm. The diameter of the base of the posterior spherical segment is 7.3 mm. These two bases give a ratio of 1:4.

In a cross-section of the eye that part of the sclera which unites these two spherical segments approximates a straight line. A slight bend occurs, but instead of curving outward, it bends inward with the convexity toward the vitreous body. The shape of this portion of the sclera would therefore closely resemble a segment cut from a funnel whose larger diameter is 7.3 mm. and the smaller, 1.8 mm. The shape of the whole eye is therefore quite different from that of man. Instead of being made up of two adjacent spherical segments, the two spherical segments are joined by a segment of a blunt cone.

The sclera is thickest where it blends with the corneal portion near the margin of the lens, where it measures about .14 mm. From this region it diminishes in thickness both anteriorly and posteriorly. In the center of the cornea it measures .071 mm. The thinnest part of the sclera is slightly posterior to the equator. At this point it measures from .03 to .04 mm. Another thickened region surrounds the optic nerve. This is thickest near the nerve and diminishes rather abruptly to almost a uniform thickness over the whole posterior part of the eye.

The sclera is pierced in several places by blood-vessels and nerves, the most important of which are the vessels to the

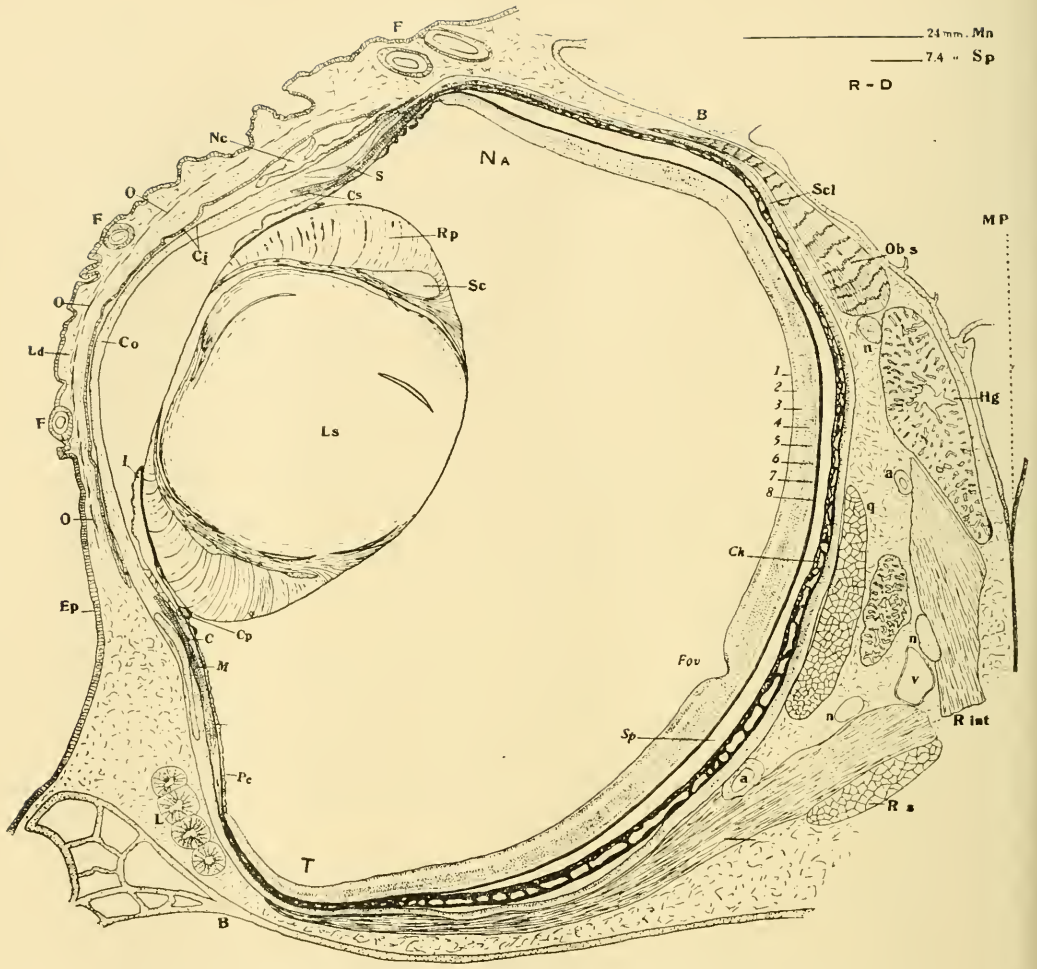


Fig. 30 Enlarged drawing of a horizontal section through the center of the lens and fovea of the eye of the adult sparrow. Outlined with camera. *1*, nerve-fiber layer; *2*, ganglion-cell layer; *3*, inner molecular layer; *4*, inner nuclear layer; *5*, outer molecular layer; *6*, outer nuclear layer; *7*, rod and cone layer; *8*, pigment layer; *a*, artery; *B*, bony portion of orbit; *Br*, location of Brücke's muscle; *C*, location of Crampton's muscle; *Ch*, chorioid; *Co*, cornea; *Cj*, conjunctiva; *Cp*, ciliary processes; *Cs*, canal of Schlemm; *Ep*, epithelium; *F*, feather follicle; *Fov*, fovea; *Hg*, Harder's gland; *I*, iris; *L*, lacrimal gland; *Ld*, lower lid; *Ls*, lenticular portion of lens; *M*, location of Müller's muscle; *MP*, median plane of head; *n*, nerves; *Na*, nasal side of eye; *Nc*, nictitating membrane; *O*, orbicularis muscle fibers of lid; *Ob s*, superior oblique muscle; *Pc*, pars ciliaris retinae; *q*, quadratus muscle; *R-D*, relative diameters of eye of man, *Mn*, and sparrow, *Sp*; *R ex*, rectus externus; *R int*, rectus internus; *Rp*, ring-like or annular pad of lens; *Rs*, rectus superior; *S*, scleral plates; *Scl*, sclera, the dotted part shows the cartilage portion; *Sc*, secretion in the lenticular cavity; *Sp*, space between the retina and the chorioid due to shrinkage in the hardening process—an artefact; *T*, temporal side of eye.

pecten and the ciliary vessels, the ciliary nerves, and the optic nerve. The blood-vessels and small nerves are described elsewhere. The optic nerve enters the eye posterior to and slightly below the center of vision. After piercing the sclera, it runs downward and forward just within the sclera, but on the outside of the retina (figs. 16 to 21, plate 4). The sclera is considerably thinner immediately over the downwardly directed nerve, thus forming a trough-like depression in which the nerve lies. Externally this extension of the nerve between the sclera and chorioid coat is not noticed. Internally, this very much elongated optic disc is covered by the pecten and cannot readily be seen.

The sclera separates readily from the chorioid coat at all points except where the blood-vessels and nerves pass through into the chorioid coat. When these are cut the sclera may be readily removed without injury to the chorioid.

The sclerotic layer of the sparrow eye is composed of closely arranged connective-tissue fibers, cartilage, and bone.

The fibrous portion of the sclerotic forms the outer portion of this layer and completely envelops the other portions. Its thickness varies at different places. Over the posterior part of the eyeball it is thinnest and varies in thickness from .004 to .010 mm. It increases gradually in thickness toward the anterior portion of the eye and attains its greatest thickness a short distance posterior to its junction with the cornea where it measures .119 mm. From this point on it becomes transparent and forms the substantia propria, or the main part of the cornea. In the center of the cornea this layer measures .054 mm. By consulting table 1 the thickness of this layer at various places may be seen.

The whole of the sclera, from the region of the lens backward, is reinforced or stiffened by a layer of cartilage. This forms the inner part of this tunic and in many places constitutes almost its entire thickness. This cartilage layer ends rather bluntly just about opposite the equator of the lens. Here it is overlapped by the scleral, or bony plates. The thickness of the cartilaginous part varies in different portions. It is in general thicker

at its anterior termination and again in a region around the optic nerve. The variation of the thickness of the cartilage layer at the anterior margin is as follows: dorsal, .032 mm.; ventral, .044 mm.; anterior, .036 mm., and posterior, .036 mm. Posteriorly from the margin it thins to from .020 to .024 mm. It maintains this thickness over the posterior surface to near the optic-nerve entrance. Here the cartilage becomes rather abruptly thickened to from .052 to .056 mm. This thickened portion forms a ring-like area around the nerve. The value of this thickened area is apparent. Owing to the very short and thick optic nerve, movements of the eyeball would cause considerable pressure in this region. This would injure the delicate structures of the retina if the sclera were not stiffer to with-

TABLE 1

Showing the thickness of the different layers of the adult cornea, chorioid coat, and sclera; also the axial and equatorial diameters of the whole eye

CONJUNCTIVA	SUBSTANTIA PROPRIA	MEMBRANE OF DESCEMET	TOTAL THICKNESS CORNEA	AT AXIS			AT ORA SERRATA			AXIAL DIAMETER OF EYE	EQUATORIAL DIAMETER OF EYE
				Chorioid	Sclera		Chorioid	Sclera			
					Cartilage	Fibrous		Cartilage	Fibrous		
0.014	0.054	0.003	0.071	0.134	0.043	0.004	0.016	0.020	0.012	6.192	7.236

stand this strain. The increased thickness of the cartilage at the anterior margin is apparently to meet the extra strain produced by the muscles of accommodation.

Imbedded in the fibrous portion of the sclera at the anterior margin of the cartilaginous layer are a number of thin plates of bone, (fig. 30, *S*) the scleratic bones, found in most birds' eyes. The number, size, and arrangement vary in different species. In the sparrow there are fourteen of these plates which have a common shape (fig. 31). They are roughly quadrilateral in outline (*C*). Their thickness is greatest in the middle portion and tapers off to a thin edge on their lateral sides. Figure 31, *D-D*, shows three of these plates cut across at right angles to the axis of vision. As can be readily seen, they overlap each other

for about one-third of their width. This overlapping is regular and shingle-like in arrangement. The combined thickness of the overlapping portions about equals the thickest portion of the plate. A ring of bone of practically uniform thickness is thus formed, completely surrounding the eye. *A B* of figure 31 shows three of these bones in their normal position, and illus-

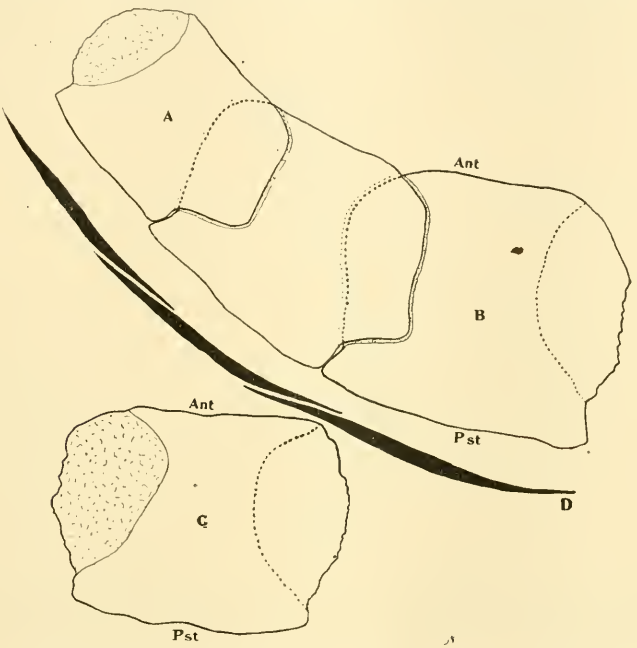


Fig. 31. Camera outline drawings of the scleral plates, showing how much they overlap and their variation in size. *A* is from the anterior or nasal side of the scleral ring; *B* from near the dorsal part; *C* is a single plate. *D* represents a section at right angles to *A-B*. *Ant*, anterior margin; *Pst*, posterior margin.

trates the extent to which they overlap. It also shows that there is a small portion at the posterior end of each plate which does not overlap. In figure 32, a free-hand drawing of an equatorial section through this region, the scleral plates (*Scl p*) are shown in their relation to the other structures.

Sections in an anteriposterior direction show the bones almost uniform in thickness and ending bluntly at the two ends. This

is shown in figure 4, *Scl.* This figure also shows that these plates are decidedly bent similar to the italic letter *f*. This bony ring, resting directly on the dense cartilage layer, gives to the front of the eye a certain degree of firmness and yet one which is capable of alteration. The ability to modify the shape of this anterior portion could be accomplished in no better way. The fact that this bony ring is made up of individual segments capable of moving on each other indicates that this arrangement

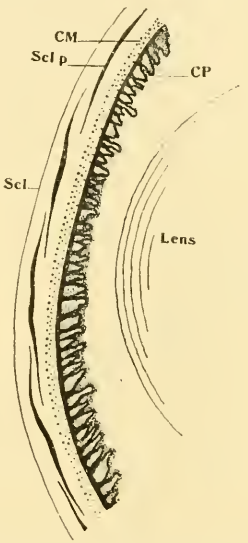


Fig. 32 Portion of an equatorial section through the ciliary region, showing the *CM*, ciliary muscles; *CP*, ciliary processes; *Scl*, sclera; and *Scl*, scleral plates. × 20.

is for some other purpose than simply that of stiffening the wall of the eye. The fact also that in sections of different eyes these plates show different degrees of bending leads one to conclude that changes in shape have occurred. These facts, coupled with the close relation of the muscles of accommodation to the sclerotic bones, lead me to conclude that their primary function has to do with accommodation. This will be discussed more in detail later.

Some differences are seen in the size of these plates. Table 2, which gives the dimensions of these bones at four different loca-

tions, shows that those at the anterior and posterior portions of the eye are smaller than the others. The anterior one is also smaller than the posterior. This is no doubt due to the fact that the eye of the sparrow is not symmetrical. Taking the axis of vision as a median line, a horizontal section of the eye shows that the two sides are not mirror-like repetitions of each other and that the lens is asymmetrically placed. Owing to the lateral location of the eyes in the head, such asymmetry tends to assist binocular vision in the bird, since that part of the retina which functions in distinct vision is more temporal. The axes of v sion will thus be more nearly parallel.

TABLE 2

Showing the dimensions of the sclerotic bones at different locations on the eye; also the distance they overlap each other at these locations. All measurements are in millimeters

LOCATION	ANTERIOPOSTERIOR DIMENSION	EQUATORIAL DIMENSION	DISTANCE EACH OVERLAPS
Anterior margin	1.022	1.600	0.512
Dorsal margin	1.408	1.856	0.586
Posterial margin	1.280	1.664	0.512
Ventral margin	1.536	1.729	0.640

THE CORNEA

The cornea consists of three principal parts: 1, the anterior layer, or epithelium; 2, the middle portion, or substantia propria, and 3, the posterior endothelial cells, or membrane of Descemet (plate 11, fig. 66).

The epithelium covering the front of the cornea is continuous with the lining of the lids and constitutes the conjunctiva. It consists of stratified epithelium. The innermost layer consists of short and broad columnar cells, The external layer is composed of very much flattened cells. Between these two layers are found the transitional forms ranging from polygonal to the flattened type. The thickness of this epithelial layer in the center of the cornea is .014 mm.

The middle portion, the substantia propria, forms the main thickness of the cornea. It is the modified portion of the con-

nective tissue or fibrous layer of the sclera. It is made up of about forty layers or lamellae of delicate parallel fibers which form a transparent whole, either free from nuclei or with nuclei which are no longer conspicuous. The thickness of this layer in the center of the cornea is .054 mm. It gradually increases in thickness toward the margins of the cornea, where it unites with the sclera, it loses its transparency and becomes opaque like that structure.

The inner endothelial layer consists of a single layer of cells .003 mm. thick. The substantia propria lying immediately adjacent to these cells is slightly more compact than the rest of the middle layer. This may be compared with a similar layer described in the eye of man as a portion of the membrane of Descemet. At the circumference of the cornea the fibers and the endothelial cells break up into bundles which bend around to join the base of the iris and the ciliary bodies. These correspond to the ligamentum pectinatum, and the open spaces between the bundles to the spaces of Fontana of the eye of man.

A study of the embryonic development of the sparrow eye shows that the endothelial cells lining the posterior surface of the cornea are derived from the vascular or chorioid coat and have been separated from this layer by the formation of the aqueous chamber. The ligamentum pectinatum constitutes all that is left of this embryonic connection.

The total thickness of the cornea at its center is .071 mm.

THE CHORIOID COAT

The chorioid coat forms the middle layer of the optic capsule. It is the vascular coat and functions mainly in the nutrition of the other layers. It consists of two principal parts: 1, The chorioid proper; 2, the derived and allied structures, such as the ciliary processes, the iris, the muscles of accommodation, and the pecten.

The chorioid proper is composed largely of blood-vessels and lymph spaces connected by a delicate connective tissue containing numerous large branching pigment cells. Being abundantly

supplied with blood-vessels and lying between the retina and the sclera, it furnishes these two layers with nourishment. No blood-vessels are seen in the retina. This layer therefore must be nourished by osmosis mainly from the chorioid. The chorioid is thickest at the axis of vision and becomes gradually thinner toward the front of the eye. At the axis it measures .134 mm. and at the ora serrata .004 mm. in thickness.

The blood for the chorioid is supplied by the short, the long, and the anterior ciliary arteries. These are derived from the ophthalmotemporal artery (fig. 15, *p*, *c*, *C*, and *lc*). After piercing the sclera at several places they spread out and branch profusely over the surface of the chorioid. The smaller branches are soon hidden by the dense pigment. A group of thirteen of these arteries is shown at *C* in figure 15. This region is slightly anterior to the center of vision. The arrangement and partial distribution of these arteries is shown in figure 16. All of these branches lie in grooves on the surface of the chorioid until they have reached their finer branches and disappear from view. The entrance of most of these arteries at the posterior part of the chorioid accounts for its much greater thickness in this region. A cross-section of the chorioid shows the large blood-vessels in the outer part of the layer and the small vessels nearer the inner margin. The long ciliary arteries are derived from a single artery which pierces the sclera a short distance posterior to the entrance of the optic nerve. This single trunk divides into a number of branches, some of which are distributed over adjacent regions while others run forward on the surface of the chorioid in grooves to the ciliary region. Other arteries, the anterior ciliary, which are derived from the superior and inferior ophthalmic arteries, occasionally pass through the sclera and sclerotic bones to the chorioid and the ciliary muscles (figs. 13, *c*, and 14). These branches and the long ciliary arteries supply the ciliary processes, the muscles of accommodation, and the iris.

The nerve supply to the chorioid is derived from the short and the long ciliary nerves (figs. 21 and 22). The short ciliary nerves, after piercing the sclera in eight different places, divide

into a number of branches and supply the greater part of the chorioid (fig. 25). The long ciliary nerves, after piercing the sclera as a single branch just posterior to the optic nerve, divides into two parts, which run forward in grooves on the surface of the chorioid on the temporal side of the eye to the ciliary region (fig. 26). Here they turn almost at right angles to their anterior course, one running dorsally and the other ventrally, to encircle the eye. In their course around the eye they are imbedded in the ciliary muscles about midway of their length. They give off numerous branches to these muscles, to the iris, and to the ciliary processes, forming a close plexus of nerve-fibers. All of these ciliary nerves are very much flattened in their course over the surface of the chorioid. The grooves in which they lie, therefore, are shallow and inconspicuous in cross-section.

In a preserved sparrow eye the sclera separates very freely from the chorioid except at a few places, where the blood-vessels and nerves pierce the sclera and enter the chorioid, binding the two layers together. Another region of close attachment is near the ora serrata. Here the posterior tendinous extension of the muscles of accommodation is rather firmly attached to the sclera. This is seen in fig. 33, which represents the posterior portion of the ciliary muscle and its tendinous continuation. An abnormal separation of the chorioid and retina from the sclera has occurred. There appears to have been a firmer attachment of the tendon at its posterior end to the sclera than to the chorioid (at *T*). Farther forward the attachment to each of these layers is so strong that the muscle and tendon fibers have been pulled apart instead of separating from the chorioid or the sclera. The significance of this will be discussed in dealing with accommodation.

A short distance anterior to the ora serrata the chorioid is modified to form the ciliary processes. These vary in number from 70 to 75, the average being 72. These processes are completely covered on the inner surface by the pars ciliaris retinae. The inner columnar cells of this layer are so thin in this region that the pigment portion is very conspicuous. The surface of

the ciliary processes is therefore covered by a densely pigmented layer. The pigment cells of the chorioid are scattered through out the processes. The blood-vessels are numerous, but not so abundant as in the chorioid.

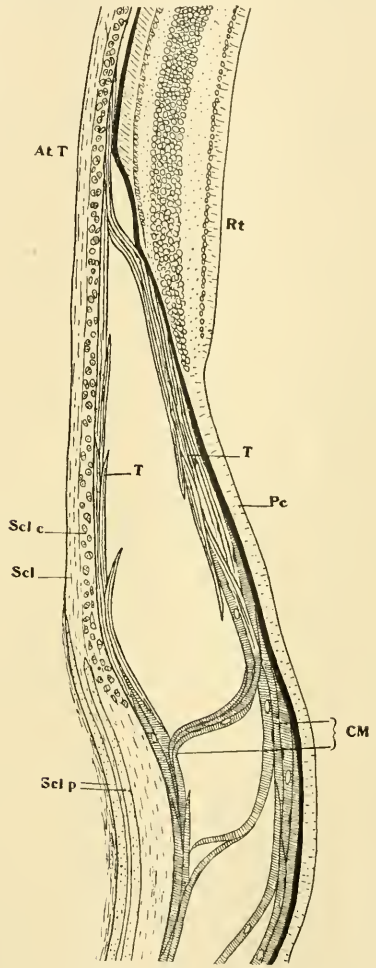


Fig. 33 Enlarged drawing from a section of an eye whose walls had parted due to stress caused by the hardening fluids. This shows a portion of the ciliary muscle, *CM*, with its tendinous continuation, *T*, attached to the sclera, *Scl*, at its posterior end, *At T*. *Scl c*, cartilage of sclera; *Scl p*, scleral plates; *Pc*, pars ciliaris retinae; *Rt*, retina.

The ciliary processes extend to the lens and are closely applied to its surface for about .7 mm. from the equator forward. The attachment of these processes to the lens is so firm that when the lens is separated from them and removed, portions of the pigment remain attached to it. Since the ciliary bodies are directly attached to the lens, a suspensory ligament is not necessary in the sparrow eye.

Still farther forward the chorioid is developed into the iris. The pars ciliaris retinae, now composed wholly of the pigmented portion or uvea, is continued forward to the edge of the pupil and forms the pars retinalis iridis, the posterior layer of the iris (plate 10, fig. 62, *uv*). At its circumference the iris is directly connected to the chorioid and ciliary processes, and by the ligamentum pectinatum to the cornea (fig. 28). According to Kölliker ('89), Schwalbe ('70) and Pflugk ('06) the fibers composing the ligamentum pectinatum are elastic. The endothelial cells of the membrane of Descemet extend from the corneal margin over the front of the iris.

The inner portion of the iris is composed of a delicate framework of connective tissue, numerous blood-vessels and nerves and muscle fibers. Pigment cells are scattered in the anterior portion. These are similar to those of the chorioid and have the same origin. The various colors of the iris found in different species of birds is due to this pigment. In the sparrow these pigment cells are brown and give the chocolate-brown color to the eye of the living bird.

The muscles of the iris consist of striated muscle fibers arranged in two layers. The anterior layer is much thicker than the posterior. It consists of circular fibers which form the sphincter muscle of the iris. These fibers are most abundant toward the circumference of the iris. Toward the margin of the pupil they are reduced to about one-fourth the maximum thickness. Toward the periphery these circular fibers become gradually less and less numerous, more widely separated, and wholly disappear at the base of the iris (plate 10, fig. 62, *cm*, and fig. 28, *C*). The posterior layer of muscle tissue, composed of radially arranged fibers one or two cells thick, is very thin.

The fibers extend from the margin of the pupil to the circumference of the iris. They lie close to the pars retinalis iridis (fig. 28, *R*). These are the dilator muscles of the iris.

There is thus a marked difference in the character of the muscles of the iris in birds and in man. Striated muscles are necessary in the bird because of the rapid locomotion. In its flight light conditions change in rapid succession. The amount of light entering the eye, which is regulated by the size of the pupil, must be regulated as rapidly as the conditions change. This could not possibly be accomplished if the muscles of the iris were composed of smooth muscle fibers as they are in man.

Steinach ('90) has made a careful study of the reaction of the iris in different vertebrates. He claims that the iris of the bird reacts only to direct stimulation. That is, each is independently stimulated, and a stimulation of one eye does not cause a reaction of the iris in the other eye. He finds this true of those vertebrates which have a complete decussation of the optic nerve-fibers. I have not verified these facts in my study of the sparrow, but I have demonstrated that the nictitating membrane of each eye does not necessarily move simultaneously. When both eyes are subjected to the same conditions the membranes usually sweep across the eyes at the same time. Numerous observations were made when one eye was exposed to bright light and the other shaded. In each case the membrane moved across the eye exposed to the light several times more per minute than that of the protected eye.

The ciliary muscles, or muscles of accommodation, in the sparrow consist of striated fibers arranged in an anteroposterior direction. Their anterior attachment is to the inner lamella of the sclera near its union with the cornea. Posteriorly, these muscle fibers terminate in a tendon which extends backward between the chorioid and sclerotic coats to the region of the ora serrata where it is attached to the sclera. From the region of the ciliary processes backward these fibers are rather firmly united to both the chorioid and sclera. This is shown in figure 33, where distortion by the preserving fluids, has caused the muscle and tendon fibers to be torn or pulled apart rather than

separate from either the chorioid or sclera. The posterior extremity of the tendon, however, shows a firm union with the sclera and a separation from the chorioid. We may infer that the separation took place at the weakest place. From this I conclude that the two attachments of this muscle are to the sclera as above stated. This conclusion is opposed to that of many other writers. Leuckart ('76) and others claim that the posterior attachment of the ciliary muscles is to the chorioid.

According to many authors the ciliary muscles of the bird are composed of three parts: Brücke's muscle, Müller's muscle, and Crampton's muscle. In the sparrow all of the fibers of the ciliary muscles run in the same general direction and are practically parallel with each other. A division into these three groups was, therefore, not accomplished. Their location, however, is shown in figures 4 and 30.

Accommodation in the bird is accomplished, in my opinion, in a different manner from that in man. Because the center of the lens, owing to its structure and great firmness, most probably cannot be changed in shape as in man, accommodation must be accomplished in some other manner. Since both the ends of the muscles of accommodation are attached to the sclera, its contraction would draw its two attachments nearer each other or exert a compressing influence on the sclera between the attachments. This would necessarily tend to bend or buckle the intervening part of the sclera. The fact that the scleral plates have been found in various degrees of bending indicates that the buckling of the sclera occurs in that region. The effect of this tension is as follows: The equatorial diameter will be reduced, resulting in increased intraocular pressure. This will cause an increase in the axial diameter and will push the cornea and lens farther forward, at the same time producing a greater curvature of the cornea. These conditions make distinct vision of a near object possible.

When the muscles of accommodation relax, the elasticity of the scleral plates would cause the sclera to spring back to its former shape and the cornea to assume its original position. The intra-ocular pressure back of the lens would be reduced,

and by means of the firm attachment of the ciliary processes to the lens, the latter would be drawn back to its passive position. Conditions are now favorable for distinct vision of distant objects. To a certain extent the process of accommodation resembles that of focusing a camera. The almost spherical lens would require very little forward and backward movement to accommodate for near and far vision.

This theory of accommodation in the bird is partly proved by the following experiment. A sparrow was anesthetized and its ciliary muscles stimulated by an interrupted Faradic current. The electrodes were applied to the eye in the region of the ciliary nerve plexus. Each time the stimulus was applied all the ciliary muscles were thrown into a tetanic contraction. With each stimulus the cornea, iris, and lens moved very noticeably forward. The lens seemed to have a greater amplitude of movement than the cornea, but the curvature of the latter was increased. When the stimulus ceased these parts quickly resumed their original positions. These observations were confirmed independently by a disinterested person called in to state what he saw when the stimulus was applied. This experiment proves that the eye of the sparrow, in the resting condition, is apparently adjusted for far vision. In other words, distant vision is a passive act and near vision requires the contraction of the muscles of accommodation.

The value of striated muscle fibers in the ciliary muscles can readily be appreciated when one considers the extremely rapid flight of the bird. The rapidity with which numerous objects confront the bird is almost beyond conception. And yet I have never seen a bird in its flight among trees and shrubbery have a collision. The power of rapid accommodation must reach such a degree of perfection in the bird as will enable it to see quickly approaching objects with sufficient distinctness to avoid collision. Such rapid changes in accommodation would not be possible were the ciliary muscles composed of smooth muscle fibers as in man.

This theory of accommodation in the bird eye is different from that advanced by most writers. Some have thought that the

pecten plays an important part in the process of accommodation by changing the intra-ocular pressure, thus pushing the lens forward. This view now has few supporters. Many try to homologize this process in the bird with that in man and claim that accommodation is due to changes in the shape of the lens.

Hess ('13), in describing the process of accommodation in the cormorant, speaks of the great change in the shape of the lens. The anterior surface shows the greatest change. This bird has a great range of accommodation, since it can pursue and catch fish under water as well as see distinctly in the air. By the use of nicotine he was able to harden the eyes and make sections showing various conditions of accommodation, in which the lens showed marked changes in shape. He claims that when the eye is at rest it is accommodated for distant objects and the lens is very much flattened; that when accommodated for near vision the axial diameter of the lens is increased, making it more spherical in shape.

Beer ('93) claims that the contraction of Crampton's muscle, because of its attachment to the inner lamella of the cornea, exerts a pull on this part which results in a backward displacement of the periphery of the cornea and an increase in curvature of the anterior surface. This reduces the tension on the ligamentum pectinatum and allows the lens, from its elasticity, to become more spherical. The increase in curvature of the lens is especially noted on the anterior surface. This would be an accommodation for near vision. When Crampton's muscle relaxes the wall of the eye would assume its original passive position, a strain would be put on the ligamentum pectinatum and the lens would be flattened. This theory is a confirmation of that advanced by Exner ('82).

A study of several hundred sections which I have of the lens of the sparrow will not support this theory. If the lens changed shape some of these numerous sections would show it. They represent about fifty individuals killed at different times and under various conditions. No change of curvature of the lenticular part of the lens can be noticed. The extremely firm lenticular part is so compact that when isolated in the fresh condi-

tion the force necessary to change its shape is far greater than is possible in the ciliary muscles. Again the only attachment of the lens is to the ciliary bodies at the annular pad. The annular pad is attached so loosely to the lenticular portion of the lens that even a slight pull would cause a separation of these two parts of the lens at the lenticular chamber rather than change the shape of the firm spherical center. The ligamentum pectinatum which joins the ciliary bodies and iris can, in my opinion, play no part in changing the shape of the lens.

After a careful study of the anatomy of the structures involved, in both fresh and preserved material, and experimentation on the living tissues, I am forced to conclude that accommodation in the bird must be accomplished in a very different manner from what it is in man. All the evidence leads me further to conclude that changes in accommodation in the sparrow are associated with changes in the axial diameter, position of the lens, and curvature of the cornea, and that these alone are sufficient to secure clear vision at different distances.

THE PECTEN

Although the pecten is not derived directly from the chorioid, it is described here because of their common origin, both arising from the embryonic mesenchyme.

In the sparrow the pecten is attached to the optic disc throughout its entire extent. Its position and relative extent is shown in fig. 1, *P*, plate 1. The optic nerve enters at *Op* and extends downward and forward to near the ora serrata, a distance of about 2.7 mm. Figure 14, plate 3, shows how near the distal, basal portion of the pecten comes to the ora serrata. It also shows the close relationship of the distal free margin to the ciliary region and the lens.

The pecten appears as a dark-brown, or black, fluted mass (Virchow, '00), completely covering the optic disc except where the nerve first enters the eye. It extends out into the vitreous body in a plane almost parallel with the path of light entering through the center of the pupil. Its widest extent in this direc-

tion is approximately 1.2 mm. The free inner margin is most deeply pigmented and appears as a median crest or ridge 1.5 mm. long to which the lateral flutes or folds are attached.

In a lateral view the shape and extent of the pecten is more easily seen (fig. 2, plate 1). Its long, curved base, following the curvature of the eyeball, is attached to the optic disc (*Op N*). Its inner free margin also shows a slight curvature corresponding to that of the base. This view shows the folds or flutes very clearly. It is readily seen that there are a greater number of folds at the base than at the free inner margin. The first fold near the optic nerve entrance extends but a short distance into the vitreous body. The second fold extends farther; the third still farther, and the fourth practically reaches the median crest or inner free margin of the pecten. Each of these four folds are broader near their base and rapidly narrow as they extend inward, until they blend with each other to form a common mass at the margin. The total number of folds at the base of the pecten of the adult sparrow is twenty. Only sixteen of these folds are of almost uniform length and extend from the base to the median crest at the inner free margin. Wood ('14) has overlooked the first two folds in his examination of this structure.

The lateral extent and shape of these folds is shown in figure 3, plate 1, a section at right angles to figure 2, along the line *A-A*. The folds are narrowed, both at the base where they join the optic disc, and at the free margin where they merge in the crest. A portion of a section taken at right angles to figure 2 along the line *B-B* is shown in figure 4. This is also shown in the microphotographs of sections in fig. 71, plate 12, and figs. 31 and 32, plate 6. The main bulk of the pecten is made up of a thin, deeply pigmented membrane which is folded on itself very much like the bellows of a camera.

A small artery (*a*) extends from a basilary artery (*ab*) along the middle of each of these folds. This basilar artery has been previously described by Mihalkovics ('73). These small arteries in the middle of each fold give rise to numerous branches which, after forming a dense capillary network (fig. 3, *c*, plate 1) empty

into two veins (*v*) at the angles of the folds. These veins flow into a larger vein which extends along the base of the pecten (*vb*) parallel with the basal artery. The general arrangement of these arteries and veins and their connection with the vessels which pierce the sclerotic is shown in text figures 34 and 35. The artery which supplies the pecten is derived from the ophthalmo temporal branch of the external ophthalmic artery (fig. 15, *p*). It pierces the sclera close to the posterior margin of the optic nerve. In its course through the sclera it takes a diagonal direction to reach the center of the base of the pecten.

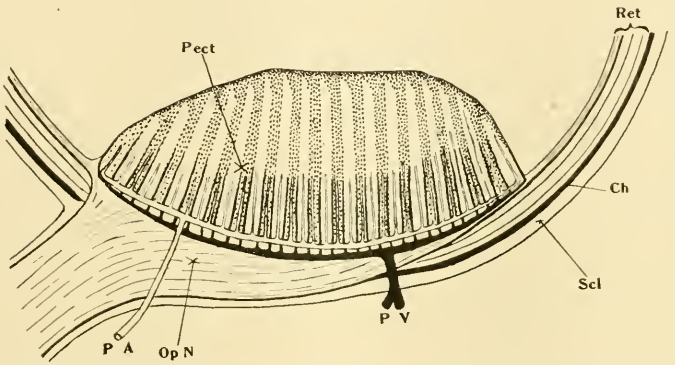


Fig. 34 Diagram showing the relations of the optic nerve entrance to the pecten and the basilar artery and vein. *Op N*, optic nerve; *Pect*, pecten; *PA*, basilar artery to pecten which sends a branch along the middle of each fold; *Ch*, chorioid; *PV*, basilar vein from pecten which receives a branch from each angle of the folds of the pecten; *Ret*, retina; *Scl*, sclera.

According to Leber ('03), the basilar artery of the pecten corresponds to the hyaloid artery of mammals; that, in the embryonic development of the bird, it does not extend out into the vitreous body, but extends along the wall of the eye. I have found this latter statement true in the development of the sparrow eye. Figure 74, plate 12, represents a section perpendicular to the retina and at right angles to the long axis of the pecten. Here the pectinal artery (*A*) is shown in cross-section at the exterior, a portion of its course through the sclera, and in cross-section at the base of the pecten. The vein (*V*) at the base is

also shown. In figure 75, plate 12, this artery is seen in a section a little farther on where the connection between its inner and external portions is not shown. When the artery reaches the base of the pecten it divides into two branches which extend in opposite directions along the base (fig. 34, *PA*). These give

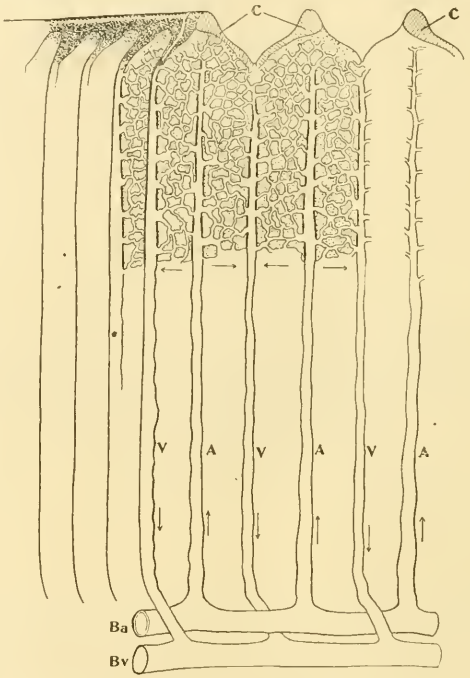


Fig. 35 Enlarged semidiagrammatic drawing from a dissection of the pecten to show the arrangement of the arteries, *A*, and veins, *V*, and their connecting capillaries, in the pecten. The crest of the folds at the free margin of the pecten has been cut at *C* to enable the folds to be spread out flat. *Ba*, artery, and *Bv*, vein at the base of the pecten. The arrows indicate the direction of flow of blood.

off branches which extend along the middle of each fold (fig. 35, *A*). The very rich capillary network is partially shown in a diagrammatical way. The ridge at the free margin is cut (*c*) so that the folds can be spread out to show the arrangement of the blood-vessels. The veins (*v*), which are situated at the angles of the folds and which are formed by the vessels from the capil-

laries, flow to the base of the pecten where they empty into the basilar vein (*Bv*). The direction of the flow of the blood in these vessels is indicated by the arrows. This basilar vein pierces the sclera a little distal to the middle of the pecten and then divides into two branches. One of the branches unites with the ophthalmic vein and the other with the ophthalmo-temporal vein (fig. 17, *P*).

The pecten consists of very loose connective tissue and pigment cells, through which the abundant blood-vessels run. No nerves can be demonstrated in the preparations at hand. I cannot, therefore, agree with those who maintain that the pecten is a sense organ (Franz, '08).

The hyaloid membrane of the vitreous body is closely applied to the surface of the pecten and entirely envelops it. Owing to the irregularity of the folds, it is rather difficult to separate this membrane from the pecten in a preserved eye. The hyaloid membrane is also closely applied to the irregular ciliary processes and, since the distal free margin of the pecten approaches close to the ciliary region (fig. 14, plate 3), one may erroneously think that the pecten is attached to the ciliary bodies or possibly to the lens. A careful dissection shows that it is only the hyaloid membrane which bridges from one to the other. In no case have I found the pecten coming in contact with the ciliary bodies or with the lens.

The pecten of the sparrow is a highly vascular organ, measuring 2.7 mm. at the base, 1.2 mm. high, and 1.5 mm. along the free margin. If the pecten were a plain body the total area of the surface would be approximately 6.8 square millimeters. But owing to the folded arrangement of this thin membrane the total area of the surface is increased to a little over 25 sq. mm. This very large surface and the rich supply of blood-vessels indicate that the function of the pecten is primarily that of nourishment. The external layers of the retina are nourished by the vessels of the chorioid. The complete absence of retinal vessels to nourish the inner layers of the relatively thick retina necessitates some other means of nourishment for this coat of the eye. The pecten contains the only blood supply from which

such nourishment could be readily obtained. This is accomplished by osmosis through the vitreous humor.

In my opinion, then, the function of the pecten is principally, if not wholly, that of nourishing the inner structures, of the eye. I cannot, therefore, agree with Beauregard ('79) or with Wood ('14) who claim that the pecten may serve as a screen to prevent the rays of the sun from injuring the retina. Since the pecten lies posterior and ventral to the point of acute vision and in a plane almost parallel with the rays of light entering the eye, it would be almost impossible for it to serve as a screen.

Neither can I agree with Abelsdorff ('98) and Franz ('08) who maintain that the pecten is a sense organ for determining the intraocular pressure during accommodation. I have found no anatomical structures which would indicate that the pecten is either a sense organ or plays any part in accommodation. My observations confirm those of Blochmann ('11), who states that the pecten consists exclusively of connective tissue and blood-vessels.

THE RETINA

The retina of the sparrow eye covers the whole of the fundus with the exception of the region of the entrance of the optic nerve. It terminates at the ora serrata near the posterior ends of the scleral plates (fig. 30; plate 7, fig. 33, and plate 8, fig. 43). If the front of the eye were cut off at the ora serrata the plane of section would cross the optic axis about two-thirds its length from the fovea and would pass through the lens slightly behind its center. The total area of the adult sparrow retina is approximately 85 sq. mm.

As in other vertebrates, a modified portion of the retina, the pars ciliaris retinae, extends forward over the ciliary processes forming the uveal pigment, and over the posterior surface of the iris as the pars iridica retinae. This portion contains no nerve-fibers.

When the front of a preserved eye is removed by an equatorial section, the retina appears as a uniform gray surface except at the optic nerve entrance and at the area centralis and fovea (plate 1, fig. 1). The optic nerve entrance is almost completely

hidden by the pecten (*P*) except a small portion where it enters. The area centralis (*A*) is a slightly thickened elliptical region with a funnel-like depression in the center which forms the fovea. It is broader in the horizontal diameter than in the vertical, being respectively 1 mm. by .8 mm. The area of this thickened region, the area centralis, is 6.2832 sq. mm. The outline of the depression forming the forea conforms in general to that of the area centralis. This is seen in the enlarged drawing, fig. 5, plate 1. The shape of the foveal depression can be made more clear by consulting plate 2, figs. 6, 7, and 8. These figures are camera-lucida outlines of horizontal and vertical sections through the fovea at right angles to the surface of fig. 5. The horizontal outline has a broader slope than the vertical The extreme bottom of the fovea forms a much sharper bend in the vertical sections than in the horizontal. This can also be observed in the microphotographs of vertical sections through the fovea (figs 24, 25, and 26, plate 5, as compared with the horizontal sections in figs. 29, and 30 of plate 6, 33 and 34 of plate 7, and 43 and 44 of plate 8.

The exact shape of this foveal depression at different levels is readily observed in camera-lucida drawings of serial sections made tangential to the retina in this region. This would be the same as sections passing through figure 6 perpendicular to the drawing and parallel to the line *H-H*.

Figure 9 *A*, plate 2, represents a tangential section at the extreme bottom of the foveal depression. Its horizontal diameter is .016 mm. and its vertical, .008 mm. This has an area of 0.0001 sq. mm. The successive drawings of this figure represent sections at different levels taken .015 mm. apart. The last one, fig. 9, *M*, represents the shape at the inner margin of the retina or at the beginning of the depression. It measures in a horizontal direction .432 mm. and in a vertical, .300 mm. The general shape of the foveal depression is that of a truncated, elliptical funnel with trumpet-shaped wall, whose dimensions are .432 × .300 mm. at the mouth, .016 × .008 mm. at the truncated end, and .180 mm. deep. The total area of the surface of this depression is 0.0997 sq. mm. Microphotographs of

a few of these tangential sections through the fovea at different levels are shown in plate 7, figures 35, 36, 37, and 38.

Figures 29 and 30, plate 6 or figure 48, plate 9, show that this depression is due to a thinning out of all the layers of the retina except the rod and cone layer and the pigment layer. The rod and cone layer is much increased in thickness in the center of the fovea. This is largely due to the lengthening of the connections between the cones and their nuclei.

The thicknesses of the different layers in the center of the fovea and at its margin are tabulated in table 3, (p. 424). This shows that there has been a reduction from .3306 mm., the total thickness of the retina at the edge of the fovea, to .1555 mm., the thickness at the center of the fovea. This reduction in thickness is apparently due to a pushing to the side or a radial migration of the cells from the center of the fovea into the area. This assumption is based on a comparison of the number of cells found in equal areas in the center of the fovea and in the area centralis. This is also indicated by the slanting arrangement of the cells and their processes from the periphery of the fovea toward the center (fig. 48, plate 9). This arrangement conforms in general to that of the foveal depression.

Table 4 (p. 425) represents the number of cells of the different layers in equal areas at various regions. Owing to the fact that the dimensions of the center of the fovea are so small, it is very difficult to get a section which does not include some cells from the sloping walls. In the above table the count was made from sections of the whole eye. These were necessarily thicker than the diameter of the center of the fovea. The count of the cells in the fovea, therefore, must necessarily include some cells from the sloping walls and is somewhat larger than it should be.

According to Cajal ('94), a one to one relationship exists in the fovea between the ganglion cells, bipolar cells, and the cones. This makes possible the sharp and distinct vision characteristic of the fovea. Clearness of vision in the fovea is also assisted by the thinning of the retina in this region. This allows the rays of light to reach the cones with less obstruction than in other portions of the retina.

From experiments on man we must conclude that distinct vision is confined to the area in the bottom of the fovea and that the clearness of detail grows rapidly less toward the ora serrata. Applying these facts to the sparrow, we find that the surface which would function in sharp vision is an ellipse .016 mm. long and .008 mm. wide. This is equivalent to an area of .0001 sq. mm. Rays of light passing through the focal center of the lens to the margins of this area would form an angle of .23 degrees in a horizontal plane and .115 degrees in a vertical plane. These angles would give as a visual field at a distance of one meter an ellipse 4 mm. broad and 2 mm. high. This is equivalent to 6.28 sq. mm. Since there are between thirty-five and forty cones in this foveal area involved, it follows that each square millimeter, one meter from the sparrow, is capable of stimulating approximately six cones. The distance at which the sparrow usually selects its food may be placed at 10 cm. At this distance each square millimeter would stimulate the equivalent of sixty cones. Taking into consideration the one to one relationship advanced by Cajal, we see that the mechanism for distinct vision in the fovea has reached a high degree of perfection.

There is a great variation in the thickness of the retina at different places. It is thickest in the area centralis at the margin of the foveal depression, where it measures .3306 mm. From this elliptical region it grows gradually less and less toward the ora serrata, where it is thinnest and measures .1280 mm. The retina at the ora serrata is thus reduced to almost one-third its maximum thickness at the area centralis.

Table 3 shows that this reduction in thickness is due to a gradual thinning of all the layers of the retina. An apparent exception to this general statement is seen in the greater thickness of the ganglion cell layer at the ora serrata than at the area centralis. As a matter of fact, the number of cells involved in these two regions is as 1 to 50. At the ora serrata this layer is only one cell deep and the cells are far apart, while at the edge of the fovea they are arranged close together and it is five cells deep. The structure which makes this layer so thick at the ora serrata is largely supporting tissue and modified retina.

Some of the layers of the retina as they are traced to the ora serrata show a greater amount of thinning than the others. The inner nuclear layer shows the greatest reduction. At the periphery it has thinned to almost a sixth of its greatest thickness. The nerve-fiber and outer molecular layers are represented by approximately one-fourth; the inner molecular layer by about one-third, and the outer nuclear, rod-and-cone, and pigment layers by about one-half their greatest thickness.

The reason for this reduction in thickness of the different layers of the retina toward the ora serrata can be readily per-

TABLE 3

Showing the thickness in mm. of the layers of the adult sparrow retina at different regions

REGION	NERVE FIBER LAYER	GANGLION CELL LAYER	INNER MOLECULAR LAYER	INNER NUCLEAR LAYER	OUTER MOLECULAR LAYER	OUTER NUCLEAR LAYER	ROD AND CONE LAYER	PIGMENT LAYER	TOTAL THICKNESS OF RETINA
Center of fovea.....	0	0.0121	0.0108	0.0245	0.0082	0.0184	0.0489	0.0326	0.1555
Edge of fovea (0.-326 mm. from center of fovea)..	0.0163	0.0245	0.0653	0.1305	0.0163	0.0245	0.0245	0.0287	0.3306
Area centralis (0.-816 mm. from center of fovea)..	0.0163	0.0243	0.0652	0.1142	0.0163	0.0163	0.0204	0.0244	0.2974
Ora serrata.....	0.0040	0.0360	0.0240	0.0240	0.0040	0.0120	0.0120	0.0120	0.1280

ceived when the number of cells is considered. If the number of cells in equal areas be determined it is noticed that the relative number rapidly decreases toward the periphery of the retina. This is shown in table 4. Comparison of the thickness of the retina of the sparrow and of man shows a marked difference. The greatest thickness in the sparrow is almost twice that of man, the ratio being 1 to 1.8. This is due mainly to the great thickness of the inner molecular and inner nuclear layers in the bird. In fact, these two layers alone are almost equal to the entire thickness of the human retina. When compared to other vertebrates, we find that the retina of the bird is thickest of all;

the fish is second; man, third; reptiles, fourth, and amphibians (the frog) thinnest of all.

In the sparrow the cones far surpass the rods in number. Schultze ('67) many years ago demonstrated this to be true for day birds, while in night birds he found that the rods predominated. This has been verified by Krause ('94) and many other more recent investigators. As in man, the rods are lacking in the fovea of the sparrow. The cones in this region are also more slender and longer than in the periphery, as demonstrated by Cajal ('94). According to Fritsch ('11), the cones in the center of the fovea are no longer and often shorter than in the surrounding area centralis. I have not found this condition to obtain in the sparrow.

TABLE 4

Relative number of cells in equal areas of the different layers of the retina of the adult sparrow at the regions indicated

REGION	GANGLION CELL LAYER	INNER NUCLEAR LAYER	OUTER NUCLEAR LAYER	ROD AND CONE LAYER
Center of fovea	25	48	25	25
Edge of fovea (0.326 mm. from center of fovea)	50	270	35	20
Area centralis (0.816 mm. from center of fovea)	25	207	27	16
Ora serrata	1	12	2	2

The cones of the sparrow possess an oil droplet, which is located at the junction of the inner and outer segments. This has been described by other investigators in many other species of birds and can doubtless be said to be common to this class of vertebrates. Krause ('94) finds that this oil droplet differs in color in various species of birds. He describes red, yellow-green, orange, and blue colors. Fritsch claims that the rod-like elements also possess oil droplets. I have not demonstrated them in the true rods of the sparrow. The oil droplet of the cone is shown in figure 36, *O*. The inner segments of the cones (*I*) are slightly longer and thicker than the outer segments. As a usual thing the greatest thickness is near their external ends. This is joined by a long tapering process to the nucleus

(*N*). In the thickened portion and in contact with the oil droplet is the ellipsoid body (*El*) described by Greeff ('97) and many other investigators in other species of birds. This occupies almost half of the length of the inner segment.

The external segments of the cones (*E*) are rather broad at the base and taper gradually to a cone-shaped point. The ratio of their length to that of the inner segments is approximately 2 to 3.

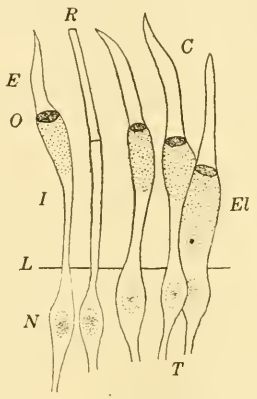


Fig. 36 Enlarged drawing of the rod and cone elements and their nuclei. *C*, cones; *E*, external segments of the rods and cones; *El*, ellipsoid body of the cone; *I*, internal segments of the rods and cones; *L*, limiting membrane; *N*, nuclei of the rods and cones; *O*, oil droplet at the junction of the inner and outer segments of the cones; *R*, rod; *T*, twin cones. × 975.

Twin cones, first observed by Hannover ('44) and described in many species of birds and in other vertebrates by numerous writers, are common in the sparrow. They consist of two cones which appear to have been crowded together so as to conform to each other's contour. No space exists between their inner segments, but their external segments usually are separated (fig. 36, *T*). A line of separation between them can usually be seen. Each has a distinct nucleus, which may lie adjacent to or separated from the other. The anatomical structures indicate conclusively that each is an independent element and that they get the name of twin or double cones because of the close relationship.

The rods are relatively few in number in the sparrow. They are much more slender than the cones (fig. 36, *R*). The relative lengths of the inner and outer segments is about the same as in the cones. Their diameter is almost uniform throughout their length and varies from one-third to one-half the diameter of the cones. The fact that the rods are more numerous than the cones in night birds indicates that they are capable of functioning in a light too dim for the proper functioning of the cones.

The rods and cones in the sparrow eye are approximately parallel to the rays of light that would pass to them through the center of the lens and not parallel to the radii from the center of the eye. Therefore, toward the periphery of the retina the rods and cones change from an arrangement perpendicular to the retina to a more and more oblique position. This is especially noticeable near the ora serrata where the rods and cones form an angle of about 28° with a perpendicular through the retina.

This arrangement enables rays of light reaching these different portions of the retina to pass through the rod and cone layer parallel to them. In other words, a ray of light coming from a given object would stimulate but one of the sensitive elements and not pass diagonally through several. This is an adaptation for distinct vision, even in the peripheral portions of the retina.

The pigment cells of the sparrow are thicker than those of man. The pigmented processes are also longer than those of mammals (Ellenberger '06). In a tangential section they appear as rather regular hexagonal bodies arranged in a honeycomb-like manner (fig. 37 *B*). In birds killed in the light the cell bodies contain little pigment. This fact was demonstrated long ago in other vertebrates by Angelucci ('78). The nucleus is rather conspicuous and is surrounded by a slightly pigmented cytoplasm. The average diameter is .0145 mm. The arrangement is so definite that each cell is in contact with and surrounded by six adjacent ones. A section vertical to the retina of a bird killed in the light shows that the pigment of the cells has migrated in long streamer-like processes between the rods

and cones (fig. 37, *A*). Near the cell bodies these pigment granules are closely grouped together and form a closely compacted mass (fig. 37, *c-c A*, and *C*). Farther inward this mass divides into from six to nine bundles of closely arranged granules (*d-d A*, and *D*). As this migration continues inward these bundles divide into brush-like filaments which lie between and largely fill the spaces between the external segments of the rods and cones so as to almost completely conceal them (*e-e A*, and *E*).

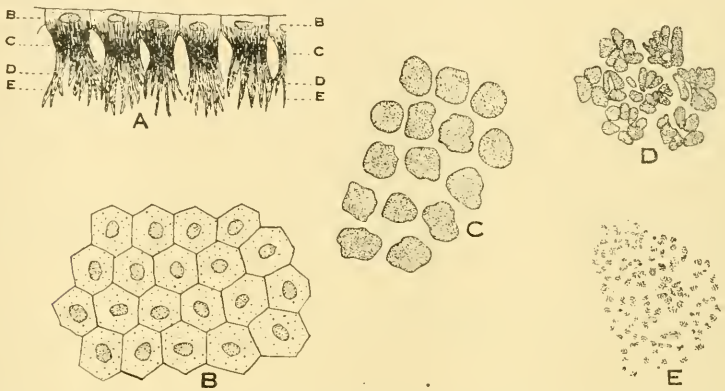


Fig. 37 Camera-lucida semidiagrammatic drawings of the pigment cells of the retina of the sparrow killed in the light. *A*, pigment cells from a section perpendicular to the retina, showing the migration of the pigment granules from the cell bodies, *B-B*, toward the inner surface of the retina. *B*, Pigment cells as seen in a section cut tangential to the retina or in the plane *B-B*, perpendicular to fig. *A*. Few pigment granules remain at the level of the nuclei. *C*, drawing from a tangential section of the eye in a plane perpendicular to fig. *A*, along the line *C-C*. This shows the pigment granules forming almost a solid mass. *D*, drawing from a tangential section of the eye in a plane perpendicular to fig. *A*, along the line *D-D*. At this level the pigment granules are arranged in bundles. E, drawing from a tangential section of the eye in a plane perpendicular to fig. *A*, along the line *E-E*. The bundles of pigment granules have further divided and now appear as minute masses which extend inward to the region of the junction of the inner and outer segments of the cones. × 465.

In the retina of a bird killed in the dark the granules show little migration and are grouped in a mass in the inner portion of the cell body. The external segments of the rods and cones are now easily seen. I agree with former writers that the function of these cells is evidently that of protecting the rods and cones from excessive light.

Since the pigment of these cells is capable of such great migration the dimension of the cell in this radial direction depends upon the condition or the amount of light entering the eye. They may vary in length from .0071 mm. in the dark to .0300 mm. in bright light. These pigment processes are arranged parallel to the rods and cones in all portions of the retina.

The combined thickness of the rod and cone and pigment layers of the sparrow is greater than that of man, the ratio being about 4 to 3.

The entrance of the optic nerve in birds is unique and requires a more detailed description than was given under the discussion of the nerve supply. The external appearance of the optic nerve as it enters the eye of the sparrow is seen in figure 23. I is elliptical in shape and pierces the sclera about .7 mm. behind and .81 mm. below the center of the eyeball as indicated by the fovea. A cross-section of the nerve a short distance from the eye is circular. Toward the eye it becomes more and more flattened until at its entrance into the eye its diameters are about as 1 to 2. The greater diameter is arranged obliquely in a downward and forward direction. Immediately after piercing the sclera the flattening continues until it spreads out over a wide area. This is especially noticeable after the removal of the sclera (fig. 16). The main part of this expansion lies just inside the scleral coat and displaces the chorioid and retina in its course. In its downward and forward course it becomes smaller and smaller by the distribution of the fibers over the retina. It finally terminates a short distance from the ora serrata (figs. 14 and 15, plate 3, and 16 to 21, plate 4). The total distance from the central entrance to its peripheral termination is approximately 2.7 mm. The fundus view shows but little of the nerve entrance (fig. 1, plate 1). The pecten covers all of the optic disc except a small oval part at the central end. When the pecten is removed the optic disc appears as an elongated area, widest at the central end and gradually tapering in its downward and forward course to a point at its termination near the ora serrata.

MUSCLE AND VISUAL TESTS

Several tests were made on sparrows to determine how nearly the bird could see directly in front, and, if possible, to determine how much the bird could converge its eyes. The left eyes of two sparrows, a male and a female, were extirpated. Recovery was rapid and inside of a week the wound had healed in a normal way. A third normal sparrow was also tested. All these birds had been kept in captivity in a large cage for a period of about five weeks.

Twenty-nine days after the operation the following experiments were made. The cage in which the sparrows were confined during the tests was situated directly in front of a south window. Black oil-cloth was spread over all sides of the cage except the one farthest from the window which was left partially open for observation. The bird was therefore in semidarkness.

Sunlight was thrown on the head of the bird while it was facing the observer and in other positions. This was accomplished by means of a mirror and consisted of flashes of short duration with intervals of rest of several seconds intervening. The bird remained at a distance of 50 cm. from the mirror during the tests. Each of the sparrows which had but one eye was given fifty tests. The results were tabulated and the angle which was made by the sunbeam and the median plane of the head was computed.

The results were very consistent and showed no wide variations. The male bird showed a slightly greater range of variation than the female. The angle formed by the sunbeam or line of sight, and the median plane in the male ranged from 0 to 6°, in the female from 0 to 5°. The average in each of these cases was 3° and $2\frac{1}{2}$°, respectively.

One may infer from this that the sparrow can see almost directly in front of it. That this power of sight is not the most acute vision it is capable of is indicated by the behavior of the bird when the light stimulus met the eye from the side. In this case the bird turned its head so that the rays of light entered the eye practically parallel to the axis of vision. That is, so

that the fovea was involved. This was quickly followed by turning so as to almost face the stimulus as above described. It would fix its gaze apparently on the stimulating object and remain quiet. If the stimulus of sunlight were continued the bird would sit facing it until a copious secretion of lacrimal fluid would cause sneezing, swallowing, and shaking of the head.

When the strength of the stimulus was greatly reduced very different results were obtained. A small piece of white cardboard was suddenly brought into view of the bird by raising it quickly above the floor of the cage. The results were tabulated and the angles formed by the median plane and the rays of light from the card computed. This was found to be 24° in the male and 26° in the female. This is approximately the angle formed by the optic axis and the median plane.

In experimenting with the normal bird very different results were obtained. When strong sunlight was reflected onto the head, in no case could it be made to face the illuminating mirror. It continually turned, first one side, then the other, toward the stimulus in rapid succession. No fixation of vision, so easily and constantly gotten in the experiments on the birds with one eye, could be obtained from the normal bird. Neither could the copious reflex secretion of tears be elicited.

The movements of the normal bird were too quick for accurate observation, but as nearly as could be determined the stimulating rays of light met the median plane at an angle close to 25°. This coincides closely with the results gotten with the cardboard on the birds with one eye and indicates that the fovea is involved in this act of vision.

In explanation of these results three questions may be raised: 1. Is the bird able to rotate its eye in the socket sufficiently to bring the optic axes parallel? 2. Is the temporal portion of the retina capable of perceiving objects directly in front of the bird? 3. May not both of the above factors operate in bringing about binocular vision in the sparrow?

The fact that only a slight movement of the eyeball has been observed would conclusively prove that the optic axes cannot be so converged as to become parallel in the sparrow. Binocular

vision in which each of the foveae is involved is therefore impossible. It has already been stated that, in horizontal sections through the whole head, the angle formed by the median plane and the axis of vision was about 65°. This evidently represents the angle formed when the extrinsic muscles are in a resting condition. Since the foregoing experiments show that this angle in the living bird may be as small as 25°, we must conclude that the sparrow is able to rotate each eye as much as 40°. This would make the axes of vision form an angle with each other of only 50°. Whether this represents the maximum amount of convergence the sparrow is capable of I cannot say. But it does show that this bird can converge its eyes as much as 80° from the resting condition and that a portion of the retina much nearer the fovea may thus function in binocular vision than would be indicated by the prepared sections.

A further fact brought out by these experiments is that the sparrow can apparently see directly in front of it. This, coupled with the ability to rotate its eye in the socket, though not sufficient to bring the axes parallel, indicates that the third supposition is correct, that is, the sparrow is able to use the temporal part of its retina in binocular vision. That the temporal portion of the retina is more active and expends a greater amount of energy than the nasal part is indicated by the richer blood supply in the chorioid back of it.

In many other species of birds that part of the retina which would serve in binocular vision is specialized for this purpose. Former investigators (Chievitz '91, '99; Stonaker '97) have shown that these birds possess not only a fovea which functions in monocular vision like the fovea of the sparrow, but a second fovea in each eye so situated in the temporal region of the retina as to receive rays of light from a common object. This second fovea varies from a well-defined depression to one so shallow as to be scarcely noticeable. The power of acute binocular vision appears to grow less as this temporal fovea becomes more shallow. Although the sparrow does not possess a second fovea, the region of the retina where such a fovea would be located is apparently able to serve for this purpose, even though no modification of

the retina has been demonstrated at this place. We can only conjecture in regard to how distinctly the sparrow sees objects directly in front of it. It is, however, evidently sufficient to enable it to avoid collision with the ordinary objects in its flight.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

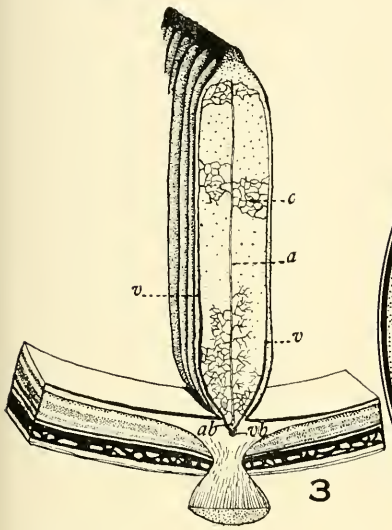
1 View of the fundus of the adult right eye as seen along the axis of vision after the removal of the anterior part by sectioning through the equatorial region. *A*, the area centralis with the fovea centralis appearing as a black dot in the center; *Op*, optic nerve entrance; *P*, pecten as seen looking down on the free edge. × 12.

2 Side view of the pecten, showing the arrangement of the folds and general shape. *A-A*, indicates the plane of section for fig. 3; *B-B*, the plane of section for fig. 4. × 35.

3 Cross-section of the pecten at right angles to the pecten through the region *A-A* of fig. 2. *a*, artery of one fold branching from the artery *ab*, at the base of the pecten; *c*, small arteries and capillaries leading from the central artery to the lateral veins of each fold, *v - v*; *Vb*, vein at the base of the pecten into which the lateral veins of each fold empty. × 39.

4 Cross-section at right angles to the pecten through the portion *B-B*, fig. 2. *a*, central artery and *v*, peripheral veins corresponding to *a* and *v* of fig. 3. × 47.

5 Enlarged view of the fovea, *F*, surrounded by its area. × 80.



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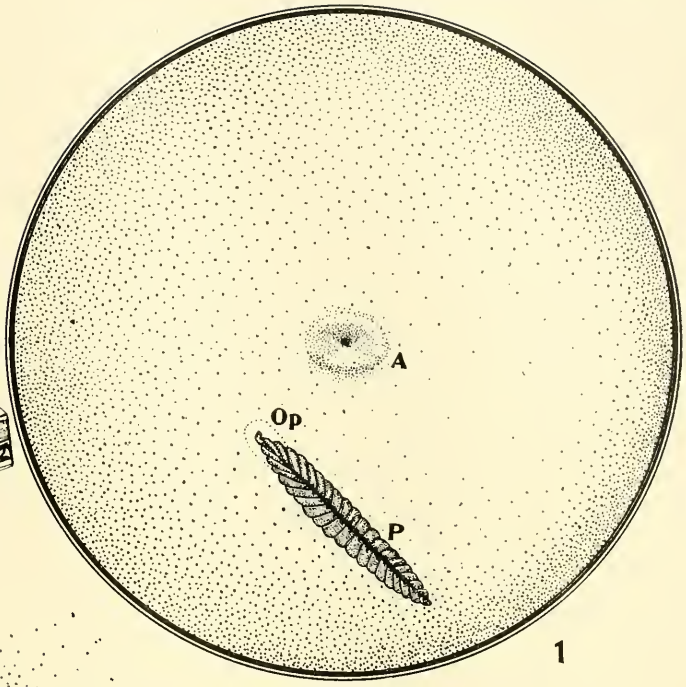

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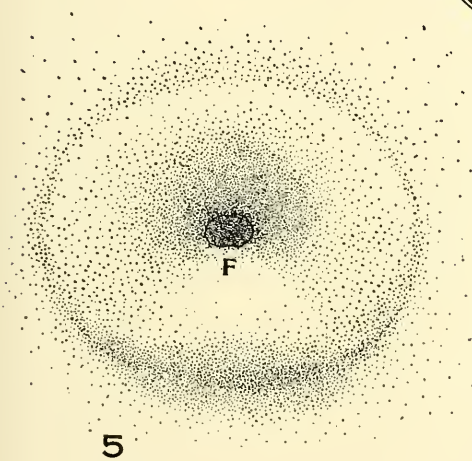

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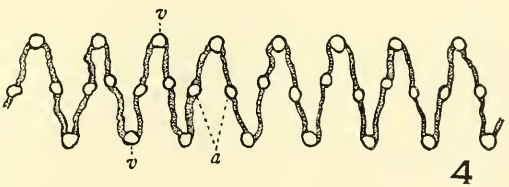

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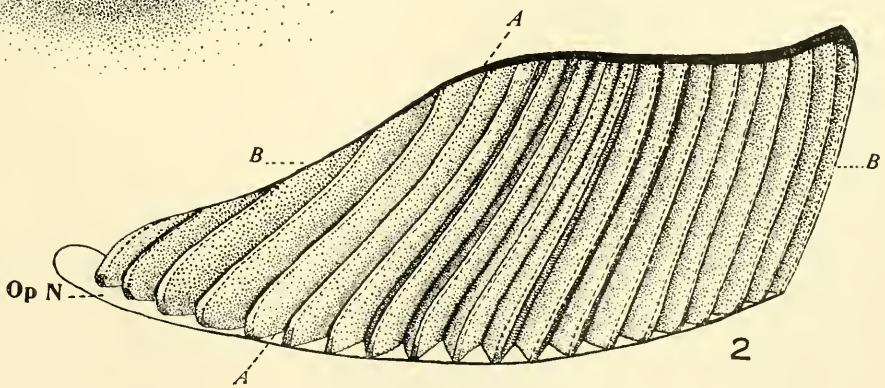

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PLATE 2

EXPLANATION OF FIGURES

Camera-lucida tracings of the outline of the adult fovea.

6 *H*, horizontal; *V*, vertical of the same specimen. × 125.

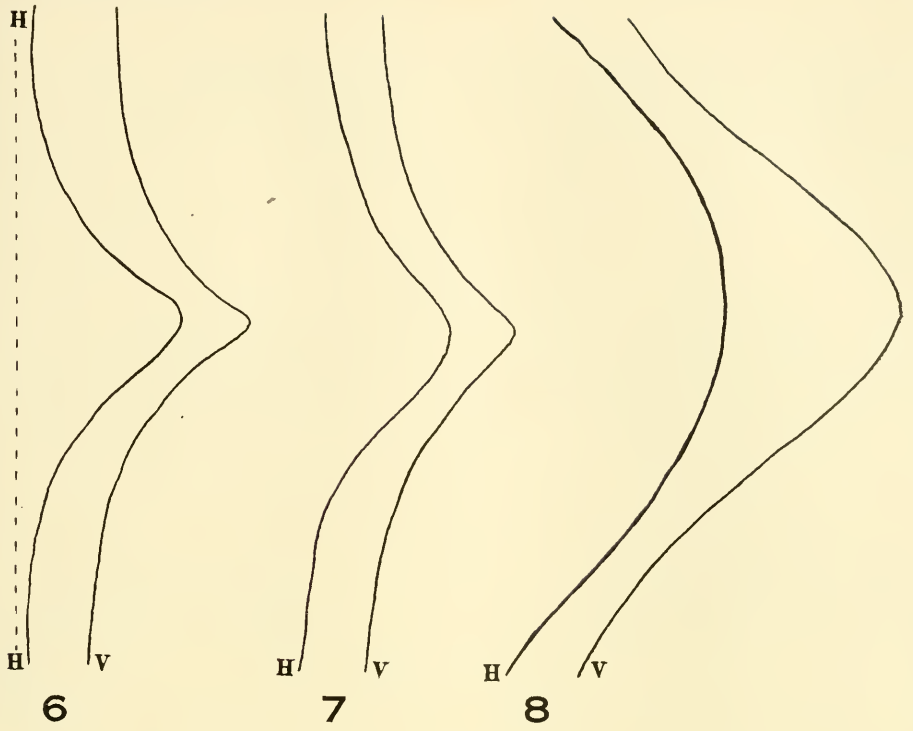
7 *H*, horizontal; *V*, vertical of a different specimen. × 125.

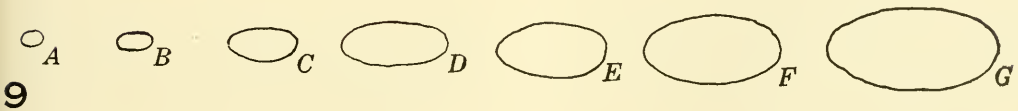
8 *H*, horizontal; *V*, vertical of the lower portion of the fovea of the same specimen as fig. 7. × 450.

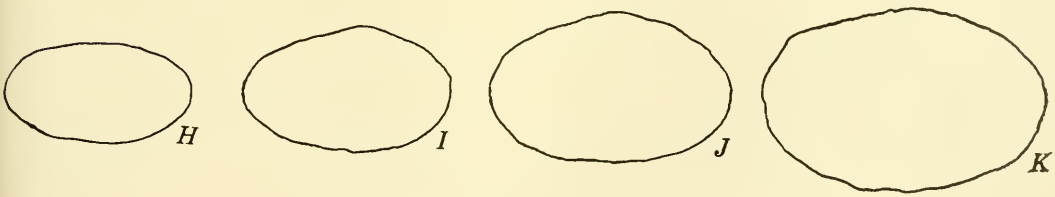
9 Outlines of the shape of the depression forming the fovea at different levels as seen in sections tangential to the retina or vertical to fig. 6, parallel to the line *H-H*. × 125.

a.	At extreme bottom of fovea.	Diam. vert. .008 mm. hor. .016 mm.
b.	.015 mm. from bottom of fovea.	Diam. vert. .016 mm. hor. .048 mm.
c.	.030 mm. from bottom of fovea.	Diam. vert. .040 mm. hor. .088 mm.
d.	.045 mm. from bottom of fovea.	Diam. vert. .048 mm. hor. .132 mm.
e.	.060 mm. from bottom of fovea.	Diam. vert. .066 mm. hor. .144 mm.
f.	.075 mm. from bottom of fovea.	Diam. vert. .088 mm. hor. .160 mm.
g.	.090 mm. from bottom of fovea.	Diam. vert. .096 mm. hor. .208 mm.
h.	.105 mm. from bottom of fovea.	Diam. vert. .104 mm. hor. .224 mm.
i.	.120 mm. from bottom of fovea.	Diam. vert. .144 mm. hor. .256 mm.
j.	.135 mm. from bottom of fovea.	Diam. vert. .176 mm. hor. .288 mm.
k.	.150 mm. from bottom of fovea.	Diam. vert. .208 mm. hor. .336 mm.
l.	.165 mm. from bottom of fovea.	Diam. vert. .234 mm. hor. .368 mm.
m.	.180 mm. from bottom of fovea.	Diam. vert. .300 mm. hor .432 mm.

This last figure is at the inner surface of the retina. The fovea in this specimen is thus .180 mm. deep and has a vertical diameter of .3 mm. and a horizontal diameter of .432 mm. at the level of the inner surface of the retina where the depression begins.


H
H
V
6
H
V
7
H
V
8


A
B
C
D
E
F
G
9


H
I
J
K

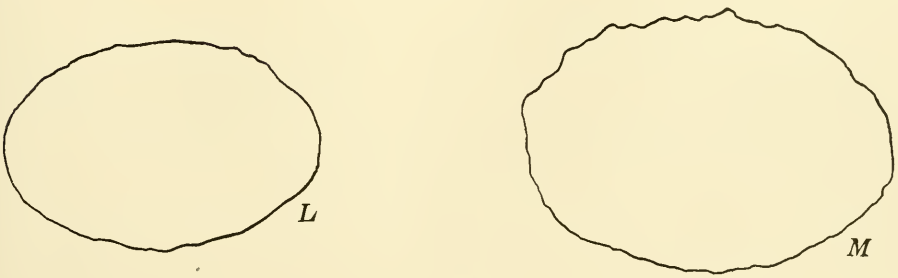

L
M

PLATE 3

EXPLANATION OF FIGURES

Microphotographs of sections through the eye of a young sparrow just after it had made its first trial flight, about twelve or fourteen days after hatching. *A*, area centralis; *Ap*, annular pad of lens; *Br*, brain; *C*, cornea; *Ca*, thickened portion of scleral cartilage surrounding the optic nerve entrance; *Ch*, chorioid; *Cm*, ciliary muscles; *Cp*, ciliary processes; *F*, fovea; *Hg*, Harder's gland; *I*, iris; *L*, lenticular portion of lens; *Lc*, lenticular chamber; *Ll*, lower lid; *Lu*, upper lid; *M*, eye muscles; *Ml*, muscle of lower lid; *N*, nictitating membrane; *Op N*, optic nerve; *Or*, ora serrata; *P*, pecten; *Pm*, free margin of pecten showing how the folds are united into a median ridge; *Py*, pyramidalis; *Q*, quadratus muscle; *Sc P*, scleral plates; *T*, tendon from pyramidalis muscle.

10 Horizontal section of left eye of young about fourteen days after hatching, section passes through the center of the fovea but to one side of the center of the lens. The fovea, *F*, shows a marked depression. × 10.

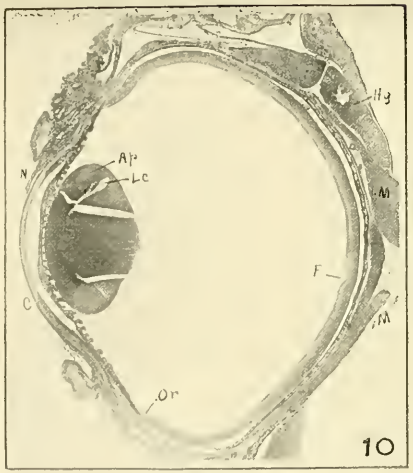
11 Same series as fig. 10 passing through the area centralis, *A*, at the edge of the fovea. × 10.

12 Same series as fig. 10 passing through the center of the lens. The lens appears as the typical adult lens. The breaks and spaces, except the lenticular chamber, *Lc*, are due to hardening and are not normal. The pyramidalis (*Py*) with its tendon (*T*) passing through the loop of the quadratus muscle (*Q*) is seen. × 10.

13 Same series as fig. 10 at a lower level some distance below the proximal portion of the nerve entrance. The free margin of the pecten (*Pm*) shows how the folds merge into a single median densely pigmented mass. The tendon, *T*, of the pyramidalis muscle is seen surrounded by the sheath-like loop of the quadratus. × 10.

14 Vertical section of the right eye of the same bird as fig. 10. Section passes through the center of the lens and the distal end of the pecten .030 mm. beyond the last trace of the optic nerve. The anterior part of the lens from which the annular pad is derived appears as a mere line. The ciliary processes are closely attached to the lens. The ciliary muscles, *Cm*, appear as a dark area lying just inside the scleral plates, *Sc P*. The muscle of the lower lid, *Ml*, can be traced back to the posterior part of the eye socket where it is closely attached to the orbital side of the inferior rectus muscle. × 10.

15 Same series as fig. 10, showing the place where the most distal part of the optic nerve penetrates the retina. × 10.



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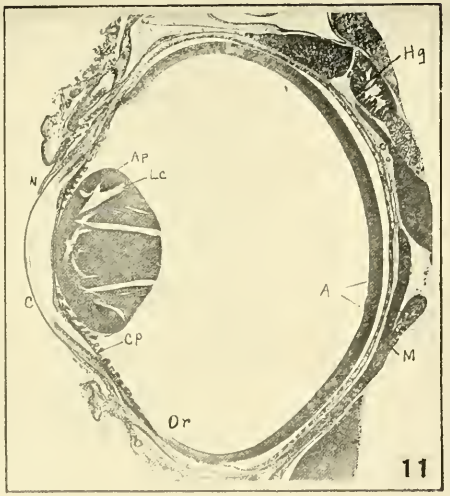

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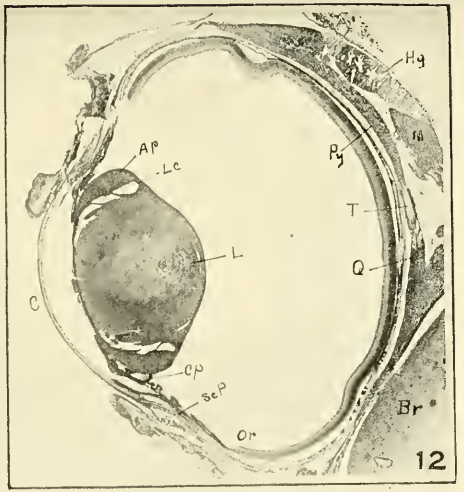

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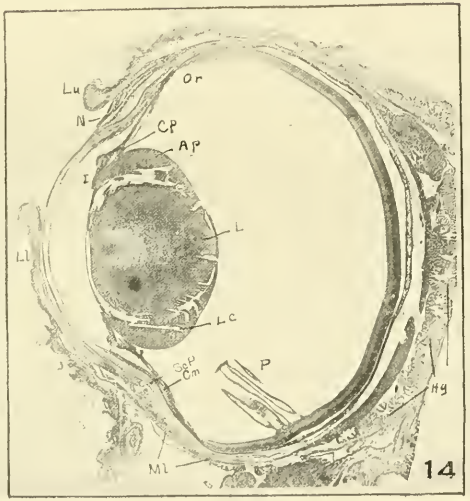
13



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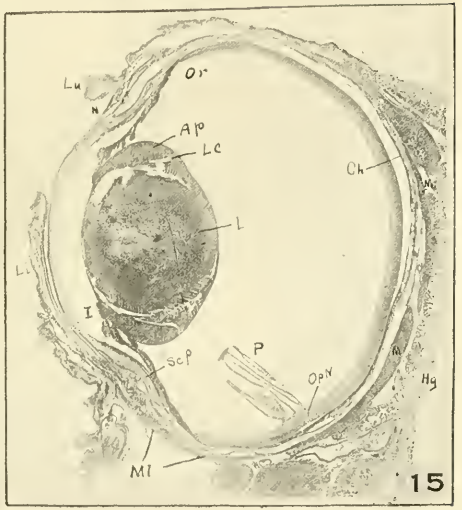

15

PLATE 4

EXPLANATION OF FIGURES

Microphotographs of vertical sections of the right eye of a young sparrow just flying, showing the arrangement and position of the nerve entrance at different levels. *Ap*, annular pad of lens; *C*, cornea; *Ca*, thickened portion of scleral cartilage surrounding the optic nerve entrance; *Ch*, chorioid; *Cm*, ciliary muscle; *Cp.*, ciliary processes; *Hg*, Harder's gland; *I*, iris; *L*, lenticular portion of lens; *Lc*, lenticular chamber; *Ll*, lower lid; *Lu*, upper lid; *Lm*, muscle of lower lid; *M*, eye muscles; *N*, nictitating membrane; *Op N*, optic nerve; *Or*, ora serrata; *P*, pecten; *Sc P*, scleral plates; *Scl*, sclera.

16 Section showing the distal end of the optic nerve piercing only the retina. × 10.

17 Section showing the distal end of the optic nerve piercing the retina and chorioid. × 10.

18 Section showing the optic nerve lying just under the sclera. × 10.

19 Section showing the thickened scleral cartilage, *Ca*, passing over the optic nerve. This portion of the nerve still lies within the sclera but pierces both the retina and chorioid. × 10.

20 This section shows the optic nerve piercing the retina, chorioid, and the thickened scleral cartilage, but is still covered by the outer membranous part of the sclera. × 10.

21 Section nearer the proximal entrance of the optic nerve. At this point the nerve pierces all the coats of the eye except the outer membranous portion of the sclera which is extended out about the nerve forming its sheath. × 10.

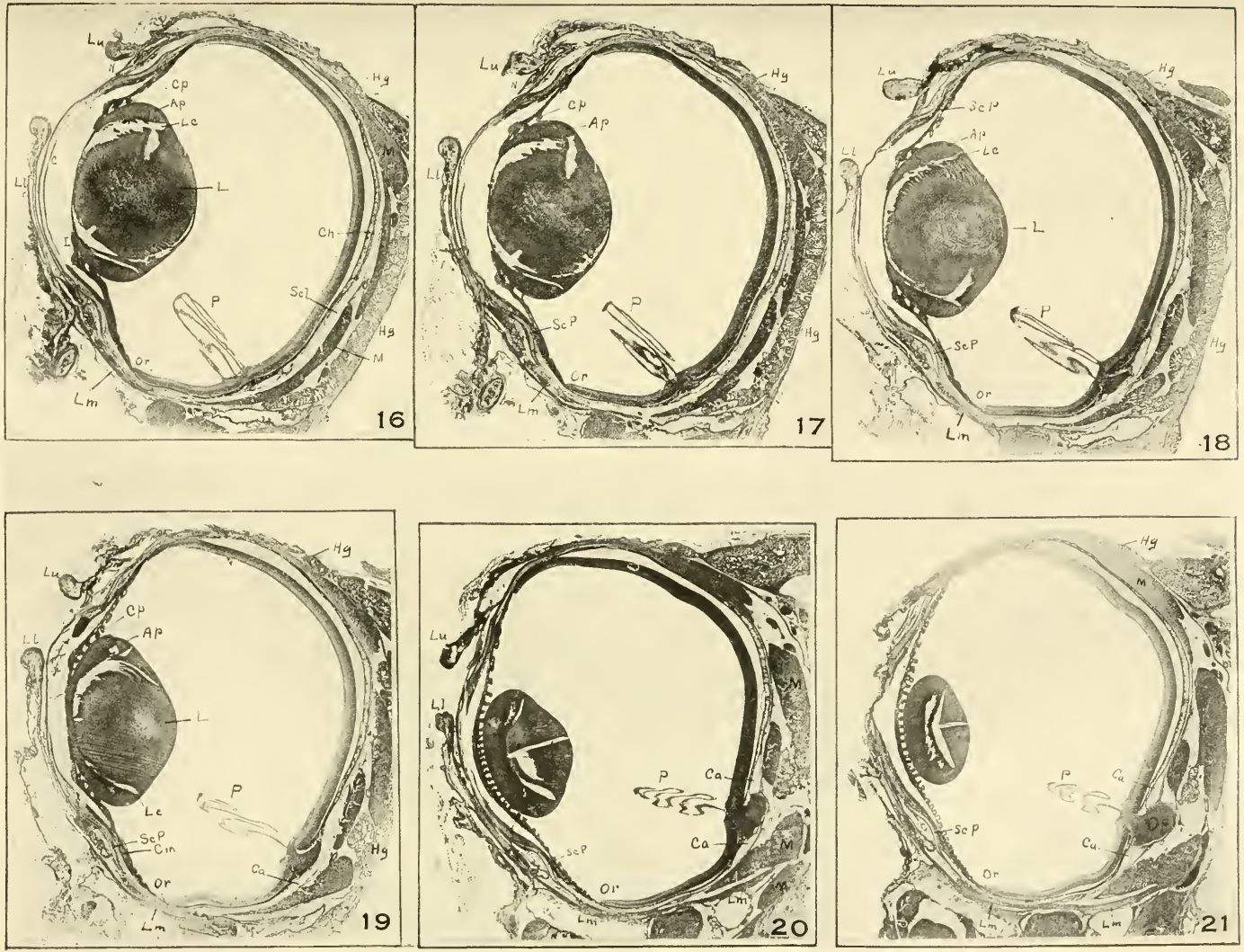
Lu
Cp
Ap
Le
L
Hg
M
Ch
Scl
P
Or
Lm
16
ScP
17
18
19
Ca
20
21

PLATE 5

EXPLANATION OF FIGURES

Microphotographs showing various structures of the eye of the sparrow. *Ap*, annular pad of lens; *Art*, artefact due to hardening; *C*, cornea; *Ca*, thickened portion of scleral cartilage surrounding the optic nerve entrance; *Ch*, chorioid; *Cp*, ciliary processes; *F*, fovea; *Hg*, Harder's gland; *I*, iris; *Lc*, lenticular chamber; *Lns*, lens; *Ll*, lower lid; *Lu*, upper lid; *M*, eye muscles; *Ml*, muscle of lower lid; *N*, nictitating membrane; *Op N*, optic nerve; *P*, pecten; *Q*, quadratus muscle; *Scl*, sclera; *T*, tendon of pyramidalis muscle in loop of quadratus. Retinal layers; *Pi*, pigment; *R-C* rods and cones; *On*, outer nuclear; *Om*, outer molecular; *In*, inner nuclear; *Im*, inner molecular; *Gc*, ganglion cells; *Nf*, nerve-fiber layer.

22 Vertical section of right eye of young sparrow just able to fly. Section passes to one side of the lens, but through the proximal entrance of the optic nerve. A portion of the outer layer of the sclera is diverted about the nerve to form its sheath. × 10.

23 Vertical section of an adult eye passing through the center of the lens. The thin movable lower lid (*Li*) is seen to extend well up over the front of the eye. The open spaces in the lens are artefacts. × 10.

24 Same series as fig. 23. Section passes through the center of the fovea. The fovea is typical for this bird. × 10.

25 Fovea of fig. 24. × 18.

26 Fovea of fig. 24. × 33.

27 Horizontal section of adult eye showing typical shape of lens and relations of the different parts. × 10.

28 Lens of fig. 27 more highly magnified to show the arrangement of the cells of the annular pad. The spaces are artefacts due to hardening. The lenticular chamber, *Lc*, more or less filled with granular-like substance is seen at either side between the annular pad, *Ap*, and the lenticular portion of the lens, *Lns*. × 20.

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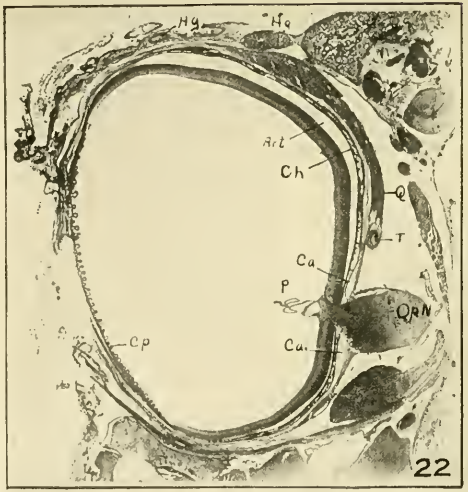
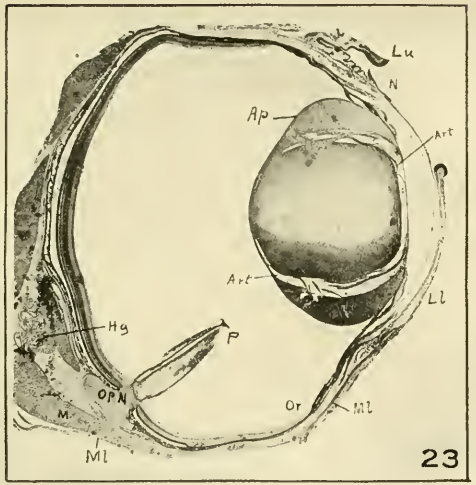
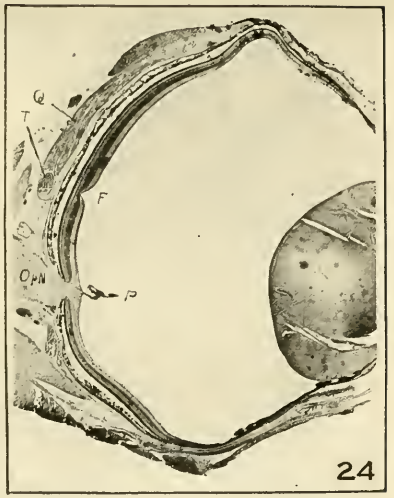
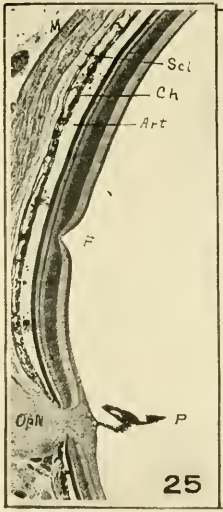
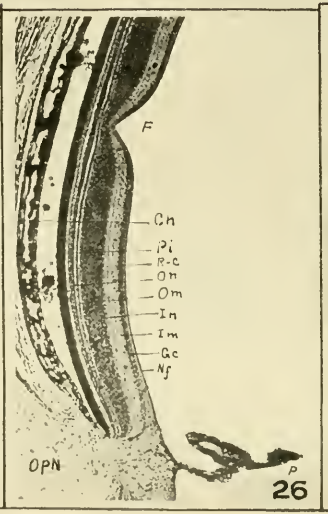
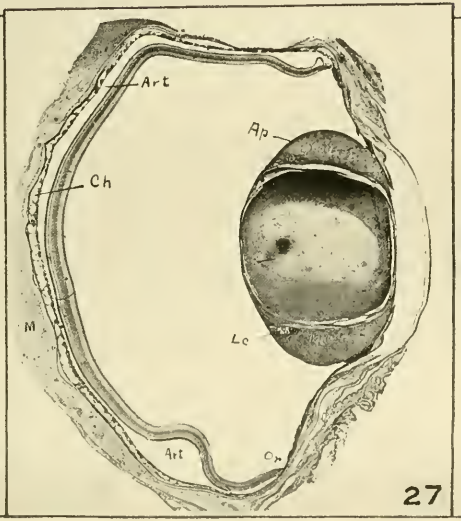












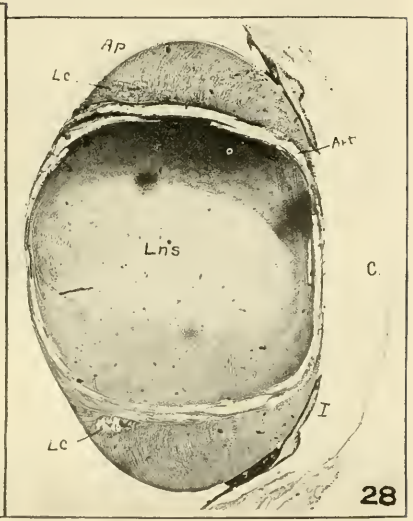


PLATE 6

EXPLANATION OF FIGURES

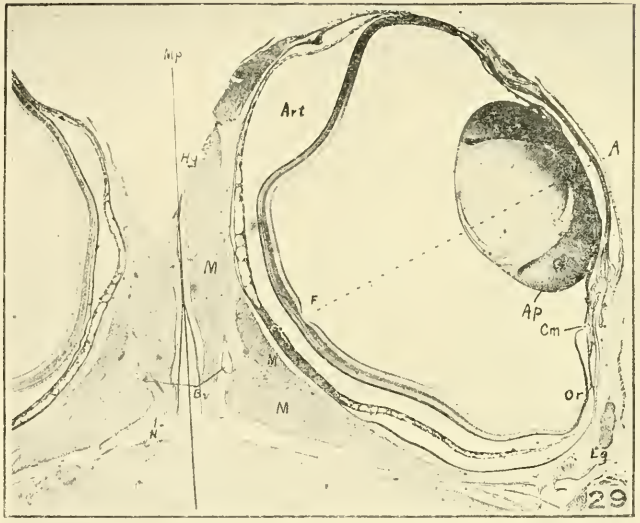
Microphotographs of horizontal sections through the head of the adult sparrow, showing the relations of the eye to the socket, the nerve entrance and optic chiasma, pecten, fovea, and the different parts of the eye. *AF*, axis of vision; *Ap*, annular pad of lens; *Art*, artefact, space due to hardening; *Bv*, blood-vessels of socket; *Ch*, chorioid; *Chs*, chiasma; *Cm*, ciliary muscles; *F*, fovea; *Hg*, Harder's gland; *Lc*, lenticular chamber; *Lg*, lacrimal gland; *M*, eye muscles; *Mp*, Median plane of head; *N*, nerves of orbit, *Op N*, optic nerve; *Or*, ora serrata; *P*, pecten.

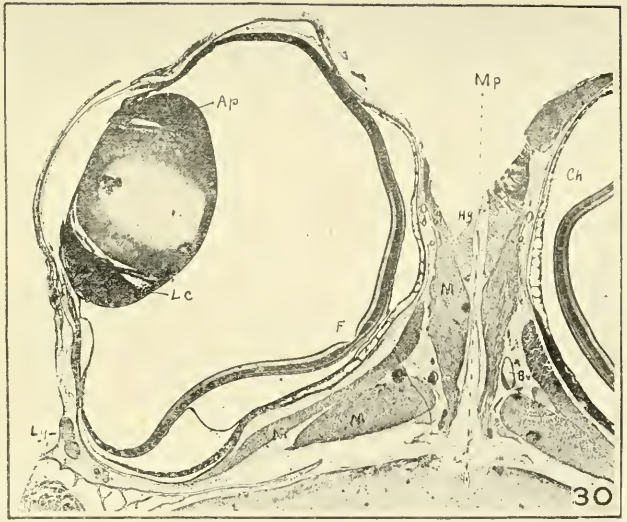
29 Section through the center of the fovea of the right eye. The section passes a little to one side of the center of the lens.

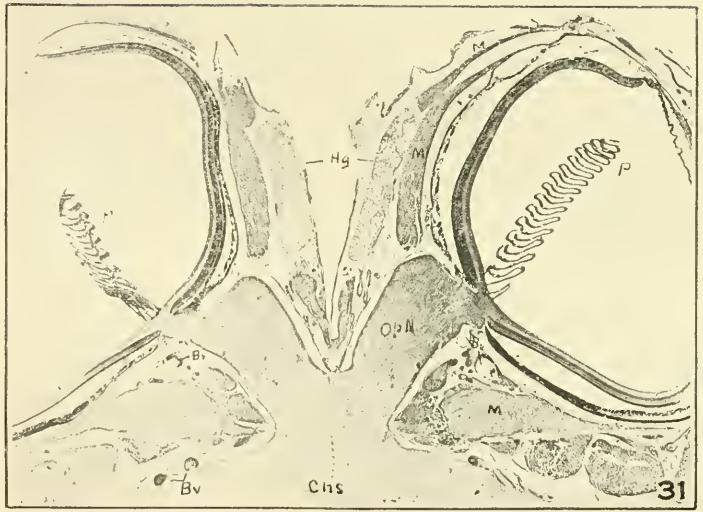
30 Section through the center of the fovea and lens of the left eye.

31 Section at a lower level passing through the nerve entrance, pecten, and the chiasma.

32 Section at a still lower level showing the proximal insertion of the internal and external rectus muscles at *M*.


Mp
Art
A
Hy
M
F
Ap
Cm
Or
Bv
N
Lg
29


Mp
Ap
Ch
Hy
Lc
F
M
Bv
Lg
30


Hg
M
P
OpN
Bv
Chs
31

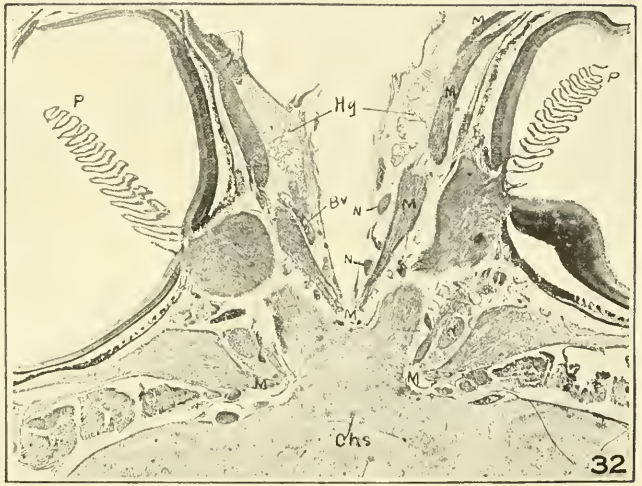

P
Hy
M
Bv
N
Chs
32

PLATE 7

EXPLANATION OF FIGURES

General explanations: *Ap*, annular pad of lens; *Art*, artefact; *bv*, blood-vessels of chorioid; *Ca*, cartilage of sclera; *Ch*, chorioid; *F*, fovea; *Gc*, ganglion cell layer; *H-H*, horizontal plane; *Im*, inner molecular layer; *In*, inner nuclear layer; *L*, lenticular portion of lens; *Lc*, lenticular chamber; *Nf*, nerve-fiber layer; *Om*, outer molecular layer; *On*, outer nuclear layer; *P*, pigment layer; *RC*, rod and cone layer; *v-v*, vertical plane.

33 Horizontal section of adult eye through the center of the fovea and pupil. × 10.

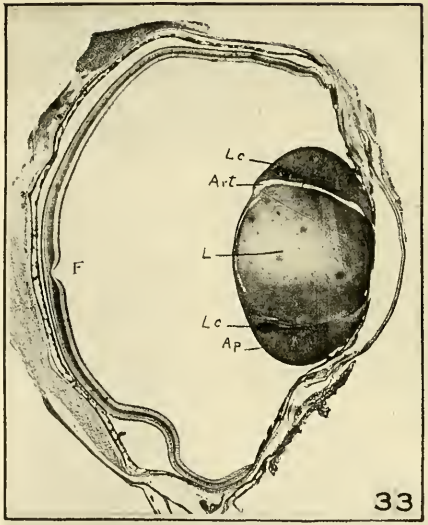
34 Enlarged view of the fovea of fig. 33. × 40.

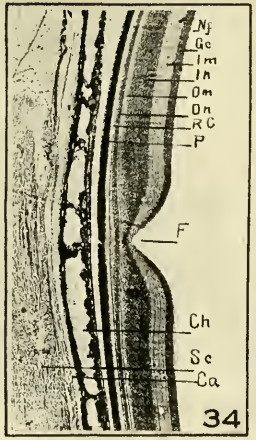
35 Section tangential to the retina through the foveal depression at the level of the inner edge of the inner molecular layer, showing some of the ganglion cells and the oval-shaped fovea surrounded by ganglion cells. × 50.

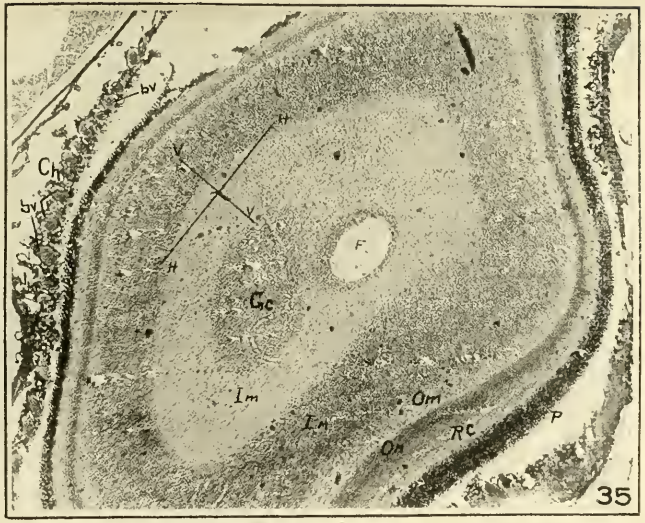
36 Section tangential to the retina through the fovea at the level of the center of the inner molecular layer. × 50.

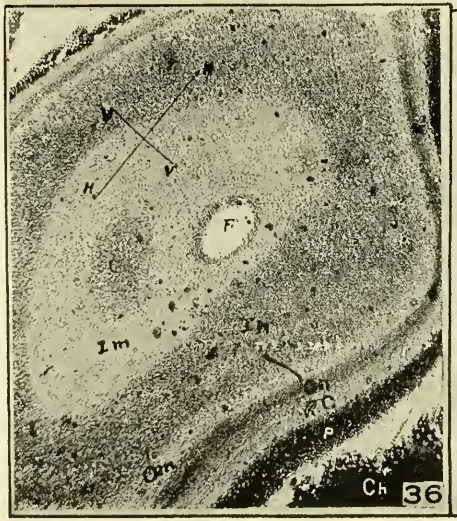
37 Section tangential to the retina through the fovea at the level of the inner part of the inner nuclear layer. × 50.

38 Section tangential to the retina through the fovea at the level of the middle of the inner nuclear layer near the bottom of the fovea. × 50.


Lc
Art
L
F
Lc
Ap
33


Nf
Gc
Im
In
Om
On
RC
P
F
Ch
Sc
Ca
34


bv
Ch
bv
H
V
V
H
F
Gc
Im
Im
Om
On
RC
P
35


H
V
V
H
F
Im
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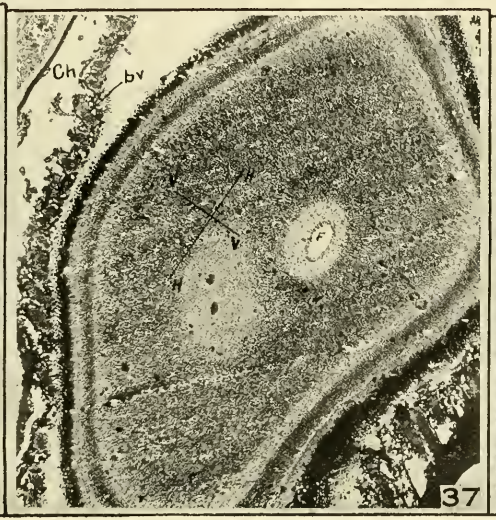
On
RC
P
Om
Ch
36


Ch
bv
V
H
V
H
F
37

bv
H
V
V
H
38

PLATE 8

EXPLANATION OF FIGURES

Microphotographs of various structures in the adult eye. *A*, artery at base of pecten; *Ap*, annular pad of lens; *art*, artefact due to hardening; *Bv*, blood-vessels of chorioid; *Ch*, chorioid; *F*, fovea; *Hg*, Harder's gland; *Lc*, lenticular chamber; *M*, muscles; *N*, nictitating membrane; *Op N*, optic nerve; *Or*, ora serrata; *P*, pecten; *Q*, quadratus muscle; *Scl*, sclera; *Scl P*, scleral plates; *T*, cross-section of tendon of pyramidalis muscle passing through loop of quadratus muscle.

39 Section tangential to the retina. The middle oval portion *C*, is the cross-section of the rods and cones. This is surrounded by a black circle representing the pigment layer. × 38.

40 Same series as fig. 39 through the bases of the pigment cells in the center surrounded by the chorioid with well-marked blood-vessels. *Bv.*, × 38.

41 Same series as fig. 39. The dark central area is a cross-section of the middle portion of the pigment cells. Surrounding this are clearer cells, which represent the basal portion of the pigment cells where they are almost free from pigment.

42 Same series as fig. 39. Through the outer portion of the chorioid showing fragments of blood-vessels. × 38.

43 Horizontal section through the center of the pupil, lens, and fovea of an adult eye. × 10.

44 The fovea of fig. 43 × 40.

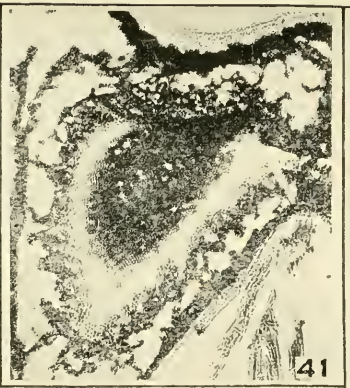
45 An oblique section of an adult eye passing through the optic nerve entrance and the fovea to show their relation. The loop of the quadratus, *Q*, and the enclosed tendon, *T*, are shown. × 20.

46 Same series as fig. 45, showing the arteries and veins of the folds of the pecten. × 15.

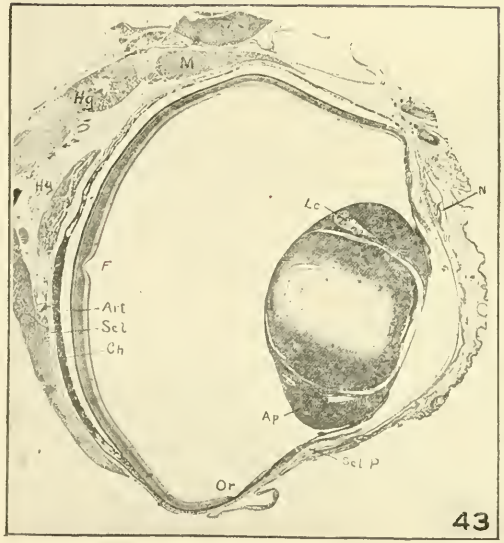
47 Same series as fig. 45, showing the artery and vein at the base of the pecten. × 15.

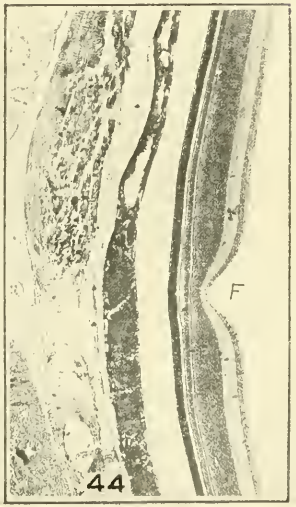
C
39

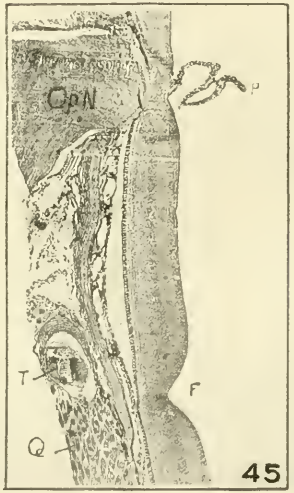
By
40


41

By
42


M
Hy
Hy
Lc
N
F
Art
Scl
Ch
Ap
Or
Scl P
43


F
44


OpN
T
F
Q
45

P
A
B
A
OpN
46

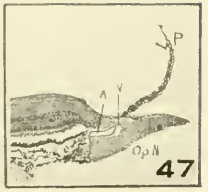

P
A
OpN
47

PLATE 9

EXPLANATION OF FIGURES

General explanations: *1*, Nerve-fibre layer; *2*, Nerve-cell layer; *3*, inner molecular layer; *4*, inner nuclear layer; *5*, outer molecular layer; *6*, outer nuclear layer; *7*, rod and cone layer; *8*, pigment layer.

48 Horizontal section through center of the fovea of adult showing relative thickness and arrangement of the layers. The cones show the marked lengthening and slanting arrangement found at the center of the fovea. × 170.

49 Section through adult eye slightly to one side of the center of fovea. × 170.

50 Section through retina of adult a short distance from fovea. × 170.

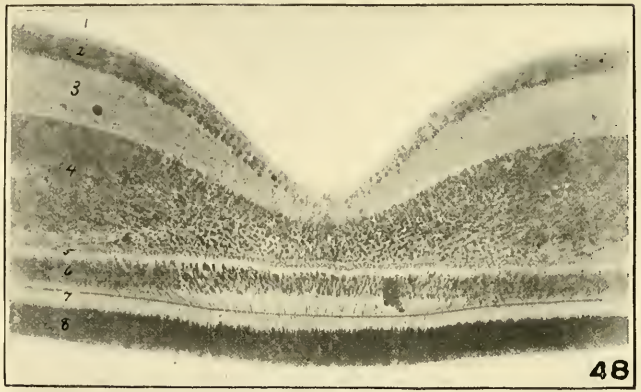
51, 52 Sections through retina of adult farther from fovea than fig. 50. Fig. 51 × 500; fig. 52 × 250.

53 Section tangential to retina of adult through the inner portion of pigment layer, showing cut ends of the rods and cones. × 250.

54 Section tangential to retina of adult through the inner segments of rods and cones. × 170.

55 Section tangential to retina of adult through outer segments of rods and cones. × 170.

56 Section tangential to retina of adult through pigment cells where the processes are in bundles. × 170.



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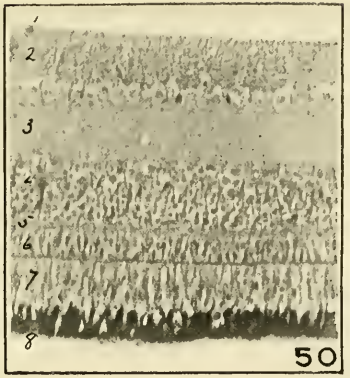

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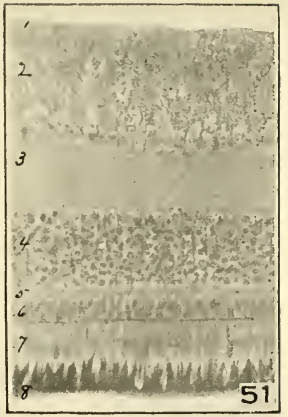

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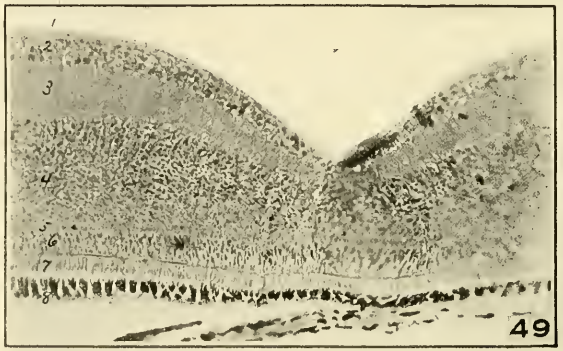

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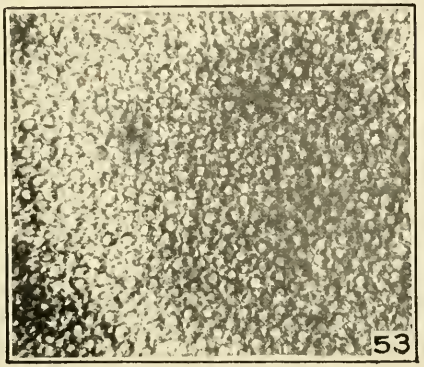
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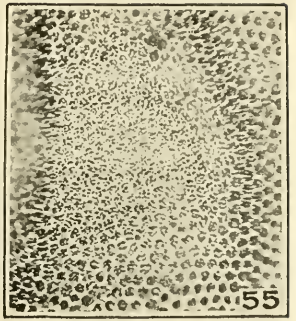
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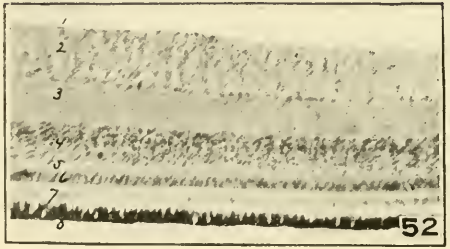

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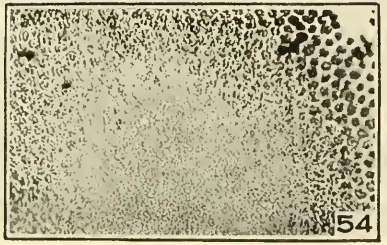
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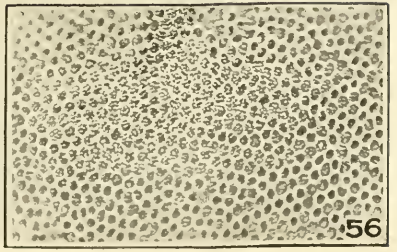

56

PLATE 10

EXPLANATION OF FIGURES

Microphotographs of different structures in the anterior part of the eye as seen in vertical section. Explanations: *Bv*, blood-vessels; *C*, cornea; *Ca*, cartilage of sclera; *Cj*, conjunctiva; *Cm*, circular muscles of iris; *Cms*, ciliary muscles; *Ep*, epithelium of lid; *F*, feather follicle; *Ll*, lower lid; *I*, iris; *Ls*, lymph space; *M*, superior and inferior levator palpebrae; *Mp*, marginal plait of nictitating membrane; *N*, nictitating membrane; *P*, pigment in stroma of iris; *Rm*, radial muscles of iris; *Sp*, scleral plates; *T*, tarsus of lower lid; *Ul*, upper lid; *Uv*, uvea of iris; figures 60 to 66 indicate the portions represented in the enlarged figures bearing these same numbers found on this and the following plate.

57 Low-power view of vertical section through the lids, cornea, and iris, showing the location of the enlarged figures represented by corresponding numbers. × 20.

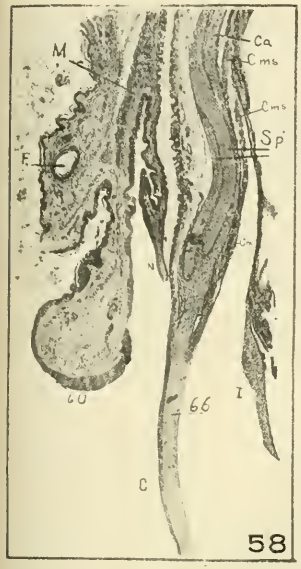
58 The upper lid and related parts. × 38.

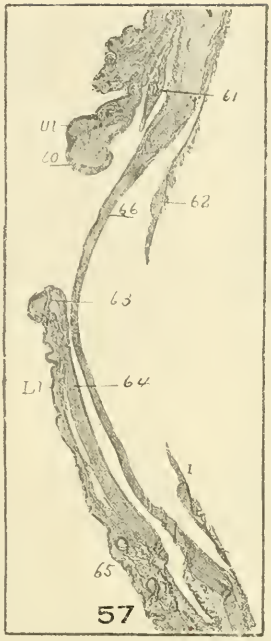
59 Lower lid and related parts. × 38.

60 Margin of upper lid as indicated in figs. 57 and 58. × 250.

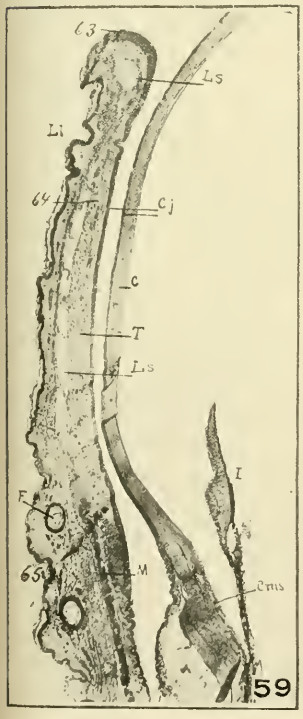
61 Nictitating membrane showing its relation to lid and eyeball. Owing to lack of distinction, the free margin of the membrane has been traced with ink. × 250.

62 A portion of the iris as indicated in fig. 57, showing the circular and radical muscles, blood-vessels, pigment, and uvea.


M
Ca
Cms
Cms
Sp
F
66
I
C
58


61
Ul
Lo
66
62
63
Ll
64
I
65
57

Cj
Ep
Ls
Bv
60


63
Ls
Li
64
Cj
C
T
Ls
I
F
Lm
65
M
Cms
59

M
Cj
MP
Cj
N
Cj
Ul
C
61

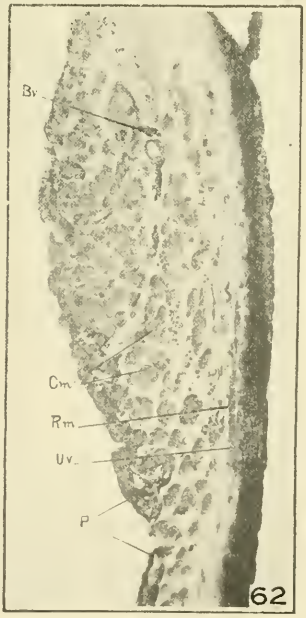

Bv
Cm
Rm
M
Uv
P
62

PLATE 11

EXPLANATION OF FIGURES

Magnified portions of anterior part of vertical section of eye as indicated in figures 57, 58, and 59. Explanations: *b*, break in the scleral plates where a blood-vessel pierces these plates. *C*, cornea; *Ca*, corneal epithelium or conjunctiva; *Cj.*, conjunctiva; *Cn*, endothelium of cornea; *Cp*, ciliary processes; *Cms*, ciliary muscles; *Ep*, epithelium; *F*, feather follicle; *Ll*, lower lid; *Ls*, lymph spaces; *M*, depressor muscle of lower lid, striated appearance shown at *M*; *Mp*, marginal plate of nictitating membrane; *Sp*, scleral plates; *T*, tarsus of lower lid, its thickness indicated by the two lines; *Ul*, upper lid.

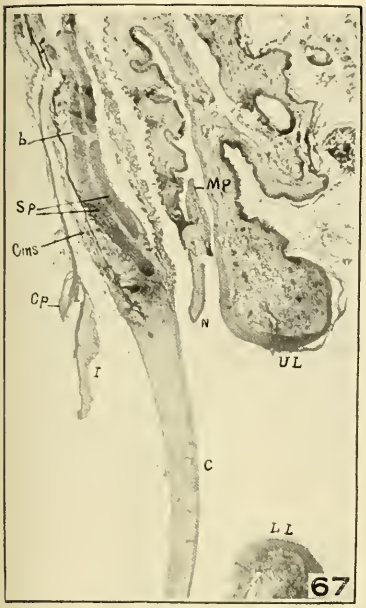
63 Magnified margin of lower lid as indicated in figures 57 and 59 at 63. × 250.

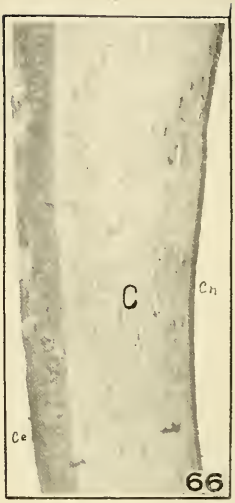
64 Magnified portion of lower lid, showing upper portion of tarsus, *T*, as indicated in figures 57 and 59 at 64. × 250.

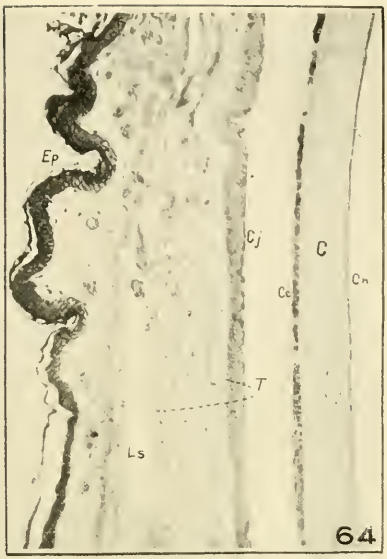
65 Magnified portion of lower lid through the depressor muscle of lid as indicated in figures 57 and 59 at 65. × 250.

66 Portion of cornea as indicated in fig. 58 at 66. × 250.

67 Vertical section through the upper lid, showing relation of parts and the sclerotic plate pierced by a blood-vessel. × 40.


b
Sp
Cms
Cp
MP
N
UL
I
C
LL
67


C
Cn
Ce
66


Ep
Cj
C
Cn
Ce
T
Ls
64

F
Cj
M
65

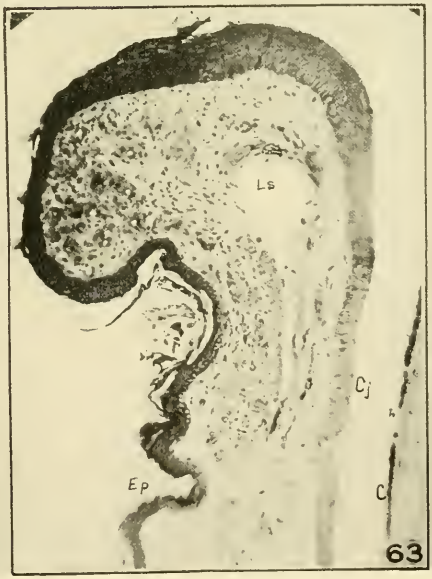

Ls
Cj
Ep
C
63

PLATE 12

EXPLANATION OF FIGURES

Microphotographs of sections through the pecten. *1*, arteries; *2*, veins of pecten; *A*, artery at base of pecten just before and after entering eye; *Op. n* optic nerve; *C*, cartilage of sclerotic much thickened at the nerve entrance; *P*, folds of pecten; *S*, sclera; *V*, vein at base of pecten.

68 Section almost parallel to the pecten and perpendicular to the retina, showing optic nerve entrance, *Op N*, and a portion of the crest of the pecten. A portion of the basilar vein is seen at V. × 38.

69 Section almost parallel to the pecten through the lateral portion of pectinal folds some distance from where the optic nerve first pierces the sclerotic. × 38.

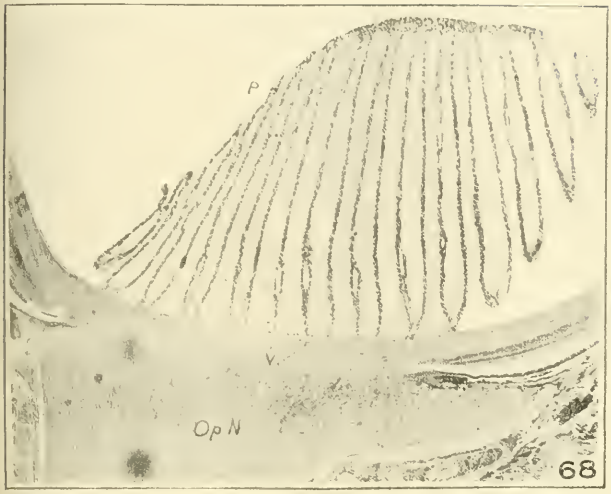
70 Section more distant from the first or primary optic nerve entrance, showing how the distal portion of the nerve lies under or within the sclerotic. × 38.

71 Section at right angles to the folds of the pecten, showing the folded arrangement and the location of the arteries, *1*, and veins, *2*. × 38.

72 Section at right angles to the folds of pecten near its base showing the basilar artery, *A-A*, and basilar vein, *V-V*, also the arteries, *1*, and veins, *2*, of the folds of the pecten. × 38.

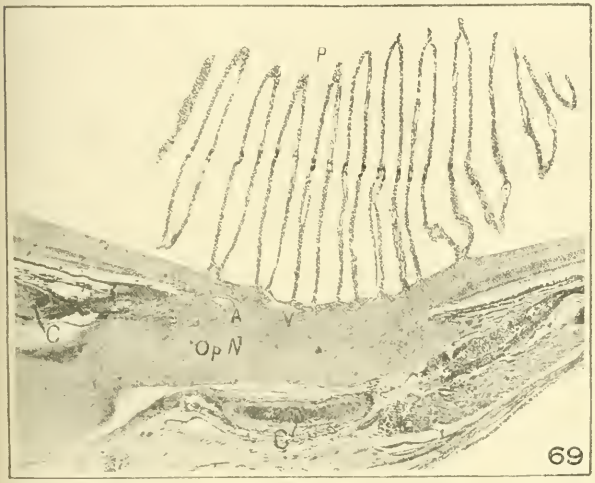
73, 74, 75 Section at right angles to optic nerve almost parallel to folds of pecten showing basilar artery, *A*, and vein, *V*, cut across.

74 Shows where the artery enters from the outside of the eye. × 38.

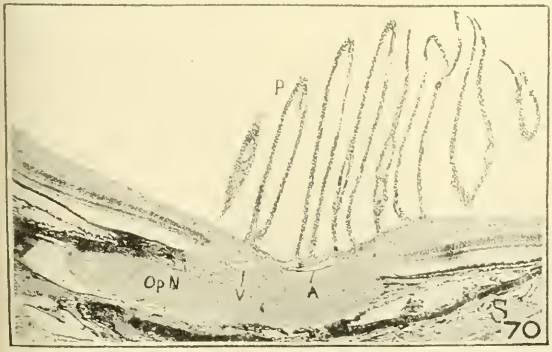

P
V
Op N
68

1
2
P
71

P
A
Op N
75


P
A
V
C
Op N
69

P
V
A
Op N
74


P
Op N
V
A
70

1
2
P
V
A
72

C
Op N
P
73

Resumido por autor, Sydney Evans Johnson.

Osteologia del pez Rhamphocottus richardsoni.

Rhamphocottus richardsoni es un pequeño pez de curiosa forma que vive en las aguas del Pacifico del Norte, desde Monterey a Sitka. Aunque esta especie no tiene importancia económica alguna, es de gran interés bajo el punto de vista taxonómico, puesto que se la considera en la clasificación actual como el único representante de la familia Rhamphocottidae. Günther, que la describió en 1874, la clasificó con los Cottidae, mientras que el Dr. Gill ('88) expresó la opinión de que debe ser colocado en una superfamilia. Estas opiniones contradictorias se basan principalmente en el exámen de los caracteres externos. Un cuidadoso exámen de los caracteres osteológicos de Rhamphocottus ha conducido al autor a la conclusión de que, aunque posee un gran número de caracteres que coinciden claramente con los de los Cottidae, existen sin embargo diferencias de suficiente importancia para elevar a este pez al rango de familia separada. Como es natural estas diferencias no pueden describirse en el presente resumen. Entre las más notables pueden mencionarse: La gran longitud del hocico, la marcada concavidad de la superficie superior del cráneo, la forma acortada, elevada y comprimida del esqueleto en conjunto y la naturaleza lisa y fibrosa de las grandes crestas óseas.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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OSTEOLOGY OF THE GRUNTFISH, RHAMPHOCOTTUS RICHARDSONI[1]

SYDNEY E. JOHNSON

Department of Anatomy, Northwestern University Medical School

TWENTY FIGURES

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I. INTRODUCTION

Rhamphocottus richardsoni is a beautiful little fish of Cottoid affinity inhabiting the waters of the eastern north Pacific from Monterey to Sitka. The species is rather abundant in Puget Sound, and a large number of specimens have been taken by the Puget Sound Biological Station dredge. Local shrimp dredgers, to whom the species is known as gruntfish or horsefish, also report getting a considerable number in their nets.

In habits Rhamphocottus is similar to the Cottidae. A rocky bottom, in shallow water, is the usual abode. Jordan and Evermann (Fishes of North and Middle America) give the range of

[1] The observations and the drawings for this paper were made in the Zoological Laboratory of the University of Washington.

depth from 2 to 10 fathoms, but in my experience on the dredge at Friday Harbor during the summer of 1913 by far the greater number was taken from a depth of 15 to 20 fathoms, and not infrequently a specimen came from a depth of 40 fathoms. The fish was rarely seen in tide pools.

Judging from stomach contents of fifteen specimens, Rhamphocottus is strictly carnivorous. So far as could be determined from preserved specimens, the food consists entirely of small stalk-eyed crustaceans; two to seven of these were found in each of the stomachs examined.

Although the fish has no economic importance, it is of peculiar interest from a taxonomic point of view, as it is, according to its present classification, the sole representative of the family Rhamphocottidae. Dr. Gill ('88) even expresses the opinion that it deserves the rank of a superfamily. Günther, who described the species in 1874, classed it with the Cottidae. In each instance, however, the classification has been based largely upon external characters. In fact, Dr. Gill had only one "moderately well preserved example" of Rhamphocottus, which, I imply, he did not dissect. For these reasons it seems desirable that the morphology, particularly the osteology, of this singular fish should be more completely worked out as a means of throwing light on its taxonomic relations. With this end in view, the writer began the work of which the following is a brief report.

My material consisted of forty specimens collected by myself during the summer of 1913 from San Juan Channel. A large collection belonging to the University of Washington was also at my disposal. The bones were dissected and cleaned under a binocular microscope after treatment with a weak macerating agent. The work was done in the Zoological Laboratory of the University of Washington in 1914, and I wish here to acknowledge my indebtedness to Prof. E. Victor Smith for criticism and many helpful suggestions.

II. OBSERVATIONS

A. DESCRIPTION OF THE SPECIES

The head is very large, constituting about one-half the length of the fish, and its greatest depth is equal to the greatest depth of the body; it has two bony ridges which above are continuous with the orbital rims and end in blunt spines at the occiput; the space between the ridges is deeply concave. The snout is notably long and narrow; the mouth is U-shaped, and its gape is longer than wide. The lips are thick, the upper lip protruding and bearing a branched dermal flap. Bands of villiform teeth occur on the jaws and the vomer; there are no teeth on the palatine bones. The eye is placed high; its diameter is contained twice in the length of the snout. Moderately long spines are developed on the frontals above the eyes. The nasal spines are strong and recurved. There is a strong sharp spine at the angle of the preoperculum, and a blunt one at the posterior angle of the operculum. A small sharp spine is borne on the posttemporal just above the operculum, and a very strong spine arises from the clavicle, just above the gill opening. The gill opening is placed high and its length is equal to that of the snout. The dorsal fins are VIII, 13; pectoral 16; ventral I, 3; anal 7; and caudal 11. The highest dorsal spine is contained nine times in the length of the body; the highest ray, six times; the longest pectoral, three times; the longest caudal, four and one-eighth; the longest anal, four and one-half; and the longest ventral, three and one-fourth times. A variable number of irregular black spots appear near the base of the dorsal spines and rays.

The color markings of the fish are characteristic as well as beautiful. A single dark stripe passes forward and downward over the side of the peduncle. Parallel in general with this stripe are five more or less complex bands arranged in loops and curves on the side of the body between the peduncle and the shoulder girdle. There is a dark patch over the lower surface of the clavicle which extends from the lower pectorals to the gill covering. A small dark ring crosses the apex of the body near the middle of the spinous dorsal and a very small dark ring may

be seen above each occipital spine. Small dark patches are arranged radially around the orbit. On the tip of the lower lip is a dark blotch, and fine black dots line the under edge of the mandible. The superior surface of the head is covered with dark spots and wavy bars to within a short distance of the occiput (fig. 1).

B. INTEGUMENT

The skin is relatively thick and tough. It is everywhere covered with minute papillae which are smaller and more numerous on the head than on the body. On the body there are approximately 400 papillae to the square centimeter, and they range from 0.5 to 1 mm. high about by 0.2 mm. in diameter.

The scales are represented by multipointed spinous processes, each arising from a polygonal bony plate which is buried in the skin beneath a papilla. Near the tip of the papilla the spinous process divides into a number (two to five) of sharp prickles which pierce the distal end of the papilla (figs. 2 and 3).

C. SKELETAL SYSTEM

1. Cranium

The *basioccipital* (*bo.*) is expanded posteriorly to form the deeply concave body of the occipital condyle. On its ventral side is a deep keel-like process running posteriorly to articulate with a similar process of the parasphenoid. It is separated from the exoccipital by a longitudinal suture which passes forward just below the auditory capsule. Posteriorly the suture passes between the large centrum of the basioccipital and the articular facet of the occipital. The posterior part of the basioccipital thus forms a narrow part of the ventral boundary of the foramen magnum (figs. 5, 7 and 8).

The *exoccipitals* (*eo.*) are relatively large bones bearing well-developed zygapophyses and, laterally, they are perforated for the vagus nerves. The exoccipitals, together with the basioccipital, form the entire boundary of the foramen magnum. Each exoccipital bone is separated from the prootic on its side by a

suture passing dorsally across the central part of the auditory capsule to the inferior angle of the opisthotic; thence the suture passes backward and inward below the pterotic and epiotic to the median line, where a slight elevation marks the articulation of the two exoccipitals, and which, with a similar elevation along the middle line of the supraoccipital, forms a rather low supraoccipital ridge (figs. 5 and 8).

The *supraoccipital* (*so.*) is interposed posteriorly between the upper posterior edges of the exoccipitals. Laterally it articulates with the epiotics. Its anterior margin is obscured by the parietals.

The boundaries of the *parietals* (*p.*) could not be positively determined in adult specimens. Laterally they enter into the composition of the strongly developed ridges which extend from the prefrontals to the occipital spines. Dorsally they meet at their posterior extremities (fig. 5).

The *epiotic* (*epo.*) bone is somewhat pyramidal in form, the apex of the pyramid being directed posteriorly (figs. 5 and 6). This part of the bone is conspicuous in the entire specimen as a long, strongly developed spine which is continuous anteriorly with the supraorbital ridge. The posterolateral margin of the bone articulates with the superior border of the posttemporal (figs. 7 and 8).

The *pterotic* (*pto.*) is irregularly triangular in outline. The base of the triangle is produced laterally as a narrow ridge which runs generally parallel with the occipital ridge. A minute canal traverses this ridge, and ventrally, near the anterior end of the bone, is a deep groove for articulation of the posterior part of the head of the hyomandibular bone. Inferiorly the pterotic joins the opisthotic by an irregular suture (fig. 5).

The *opisthotic* (*opo.*) is a thin lamina of bone of rhombic form situated on the side of the cranium between the pterotic above and the exoccipital and prootic below (fig. 5).

The *prootic* (*pro.*) consists of a thin lamina, which articulates with the opisthotic and exoccipital bones superiorly and posteriorly, and a knob-like process near its anterior end which articulates with a process of the sphenotic and the dorsal limb of the

parasphenoid. The anterior end of the bone enters into the composition of the orbital rim (fig. 5).

The *sphenoid* (*spo.*) is of irregular shape and its exact boundaries could not be determined. Immediately posterior to the orbit it bears a prominent process which extends downward and unites with the anterior tuberosity of the prootic. The anterior ball on the head of the hyomandibular articulates in a socket formed by the posterior angle of the sphenotic. The bone is pierced by a relatively large sensory canal (figs. 5 and 7).

The *alisphenoid* (*als.*) is a small rectangular lamina of bone located in the posterior wall of the orbit. It articulates above with the frontal; posteriorly, with the sphenotic and the prootic, and below with the middle of the parasphenoid (figs. 5 and 7).

The *parasphenoid* (*pas.*) bone consists of an expanded body which articulates superiorly with the alisphenoid, the sphenotic, and the prootic, and an anterior rod-like process which passes forward under the orbit to articulate with the vomer and the prefrontals. Rib-like processes extend upward from the body of the bone on each side and articulate with the prootics. Posterior to these processes the central part of the bone is compressed to form a deep median keel which unites posteriorly with a similar median lamina of the basioccipital. On either side of this process are the openings of the canals which lead forward to the myodome (figs. 5 and 7).

The *frontals* (*fr.*) are wide, arched bones forming the greater part of the superior wall of the orbit. They meet in the median line where the interorbital space is strongly concave. The supraorbital ridges are prominent and each bears a small sharp spine posteriorly above the orbit (fig. 5).

Lateral to the anterior end of each frontal is the corresponding *prefrontal* (*pf.*). The distal end of each is expanded to articulate with the palatine bone. The posterior end tapers to a slender process which unites with the anterolateral margin of the frontal. Anteriorly each bone takes part in the formation of the nasal canal (figs. 5 and 6).

The *ethmoid* (*e.*) is elongated and slightly convex superiorly. From its central part a prominent process extends upward to

unite with the nasal bone. Posteriorly the bone forks broadly, the wide limbs overlapping the superior surface of the frontals. Distally the bone forks narrowly to each side of the central limb of the vomer (figs. 5 and 6).

The *vomer* (*v.*). The anterior part of the vomer is smooth, wide, and crescent-shaped and from this part a median process extends caudad to articulate with the parasphenoid and the ethmoid. The under edge of the crescentic part is beset with numerous villiform teeth (figs. 5, 6 and 7).

The *nasal* (*na.*) bones are relatively large and bear strongly developed sharp spines. Each has a groove on its anterior surface separating it in part from the ethmoid and the prefrontals. This groove forms one margin of the nasal foramen. The outer wing of the nasal articulates with the prefrontal, and the inner process, with the ethmoid (figs. 5 and 6).

2. Suborbital ring

The suborbital ring consists of three bones. The anterior or preorbital (*por.*) is wide and flat, slightly convex externally, and articulates with the prefrontal superiorly and passes down over the side of the snout. The general shape and position of this and the next two bones described are shown in figure 4. The middle member of the suborbital ring, the suborbital (*sor.*), is a thin, wide bone of irregular outline, also slightly convex externally. The bony stay or postorbital (*ptor.*) is the third and largest of the bones comprising the suborbital ring. Posteriorly it is somewhat ovoidal in shape and the outer surface is strongly convex. From its articulation with the suborbital anteriorly it passes into the anterior angle of the preoperculum where it is firmly attached (fig. 4).

3. Suspensorium, mandibles, and opercular apparatus

The *hyomandibular* (*hm.*) is a strong bone of irregular outline. The superior part is expanded to form a large head which articulates in a groove just beneath the lateral shelf of the pterotic, on its anterior half, and overlapping slightly the sphenotic bone.

The anterior arm of the hyomandibular extends downward and terminates in an irregular articulation with the symplectic and the metapterygoid. The outer or posterior fork is very strongly developed and affords support for the upper end of the preoperculum. Just posterior to this fork is a short blunt projection bearing a socket with which the anterior angle of the operculum articulates (figs. 4 and 13).

The *symplectic* (*sy.*) is a thin crescentic lamina (fig. 8) bounded on its concave anterior margin by the quadrate and the metapterygoid, and on its posterior or convex margin by the preoperculum and the hyomandibular.

The *quadrate* (*q.*) is a fairly strong bone of roughly rectangular form. The upper part is thin and membrane-like, but the lower anterior angle is thickened and produced as a rounded knob (fig. 13), which gives attachment to the articular process of the mandible (*ar.*, fig. 13). It is bounded anteriorly by the pterygoid, superiorly by the mesopterygoid and the metapterygoid, and posteriorly by the symplectic and the preoperculum.

The *metapterygoid* (*mpt.*), the *mesopterygoid* (*mspt.*), and the *pterygoid* (*pt.*) are delicate laminae of bone, the position and boundaries of which are shown in figure 13. The pterygoid is the strongest of the three; its lower part is wedge-shaped, while the superior portion is considerably thickened and curves forward, uniting by a wedge-like process with the palatine bone.

The *palatine* (*pa.*) is irregular in form, having an enlarged posterior part which articulates with the pterygoid as mentioned above, and an anterior, slender, somewhat outwardly curved process which extends almost to the vomer. The palatines bear no teeth (fig. 13).

The mandible consists of three bones (on each side), the articular, the angular, and the dentary.

The *articular* (*ar.*) is a relatively strong bone, expanded posteriorly to articulate with the quadrate and tapering to a sharp point anteriorly where it unites firmly with the dentary. A thin triangular process extends upward and outward, and a blunt process, downward, from the posterior end of the bone. The latter process gives attachment to the small and irregular angular (*an.*) bone (fig. 13).

The *dentary* (*d.*) is forked widely posteriorly; anteriorly it is curved inward to join its fellow of the opposite side. The lower limb of the posterior fork has a groove on its outer surface for reception of the wedge-shaped anterior end of the articular bone. The superior limb curves upward and its posterior part is wide and flat. The superior edges of the dentary bones are beset with rows of villiform teeth as shown in figure 13.

The maxillary apparatus consists of a pair each of maxillae and premaxillae.

The *maxilla* (*m.*) is a rather strong rod of bone, the inferior end of which is produced as a flat, paddle-like process. The superior end is expanded to form a rounded knob which is attached to the shaft of the bone by a distinctly grooved neck, which bends posteriorly at a right angle to the main part of the bone (fig. 9).

The *premaxillae* (*pm.*) are firmly united along the median line by synchondrosis. Anteriorly they form a curved expansion of bone, the under or convex surface of which is thickly covered with villiform teeth. From the expanded anterior part of the bone a slender rod of bone extends posteriorly in a groove between the nasal spines (figs. 4 and 9). In the living specimen the premaxillae are highly protractile.

The opercular apparatus is made up of operculum, preoperculum, and interoperculum. A suboperculum is absent.

The *operculum* (*op.*) is slightly convex on its lateral surface and is triangular in its general outline. The anterior angle of the bone is considerably thickened and articulates by a distinct facet with the opercular process of the hyomandibular (figs. 4 and 13).

The small and flattened *interoperculum* (*iop.*) is coossified with the inferior angle of the operculum. A slender process of the bone extends downward for a short distance, medial to the inferior arm of the preoperculum, and is connected by a slender ligament with the angle of the mandible (fig. 13).

The *preoperculum* (*pop.*) is the largest of the series. Its posterior angle is produced as a long sharp spine. The general form and connections of the bone are shown in figure 13.

4. Hyoidean apparatus

The *interhyal* (*ihy.*) is a short, slender rod connecting the epihyal with the suspensory apparatus. The lower or posterior end is expanded and makes a rather strong articulation with the superior end of the epihyal (figs. 11 and 4).

The *epihyal* (*ephy.*) is a triangular expansion of bone, the shape and attachments of which are shown in figure 11. The bone is thicker and stronger near its superior end.

The main part of the cornu is formed by the *ceratohyal* (*chy.*). The anterior margin of this bone is slightly concave while the posterior border is interrupted by an angular projection near its superior end (fig. 11).

The *hyohyals* (*hhy.*) and the *glossohyal* (*ghy.*) constitute a group of ossifications connecting the two opposite ceratohyals below.

The *branchiostegal rays* (br.) are six slender rods of bone, on each side. Their attached ends are flattened and considerably expanded. The two superior rays are attached to the outer face of the epihyal, the next two, to the outer surface of the ceratohyal, and the two inferior rays articulate with the inner surface of the ceratohyal (fig. 11).

5. The branchial arches

The *superior pharyngeals* (*s. ph.*) of each side are coossified to form two solid bones which, in turn, unite firmly along the median line. The under surface of these bones is well provided with long villiform teeth (fig. 12).

The *epibranchials* (*epbr.*), four on each side, are slender rods of bone which join the superior pharyngeals medially with the four *ceratobranchials* (*cbr.*) laterally. The latter are elongated rods which curve around the pharynx, the three anterior bones uniting with the *hypobranchials* (*hbr.*) ventrally and the posterior one with the posterior basibranchial (fig. 12).

The *basibranchials* (*bbr.*) are poorly defined and incompletely ossified. They intervene ventrally between the hypobranchials and the posterior ceratobranchials of the opposite sides (fig. 12).

The two large *inferior pharyngeals* (*i. ph.*) are united along the median line and lie in the floor of the pharyngeal cavity immediately posterior to the fourth pair of ceratobranchials. They are triangular in shape and their superior surfaces are covered with strong villiform teeth (fig. 12).

6. Shoulder and pelvic girdles

The supraclavicle (*scl.*) is a flat, elongated bone, the superior end of which is expanded into an angular process which bears two rounded processes for articulation with the under surface of the posttemporal. The inferior and more flattened part of the bone is attached to the superior surface of the clavicle (fig. 10).

The clavicle (*cl.*) is a large bone of very irregular form. It extends upward and backward from the angle of the mandible to a point just posterior to the posttemporal and almost directly beneath the occipital spine. The upper end is wide and broadly forked, the lower limb extending backward as a prominent sharp spine. Centrally the bone narrows considerably, but inferiorly it again broadens into a wide delicate lamina which curves about on itself to form free dorsal and ventral wings (figs. 4 and 10).

From the under surface of the superior end of the clavicle the long, slender, and flat postclavicle (*pcl.*) passes posteroventrally into the lateral body wall for a considerable distance (*pcl.*, figs. 4 and 10).

The upper arm of the hypercoracoid (*hpc.*) is pierced by a large foramen. Below the foramen the bone is lamina-like and lies closely against the clavicle.

The hypocoracoid (*hpoc.*) is a wide irregular bone joined closely to the posterior margin of the clavicle without the intervention of foramina (fig. 10).

Three or four wide, lamina-like mesocoracoid (*mc.*) bones occupy the wide space between the actinosts, hyper- and hypocoracoids. Their exact number could not be positively determined because of their membranous character and their indefinite boundary lines (fig. 10).

The actinosts (*ac.*) are flat, rectangular bones, four in number, to the outer edges of which most of the pectoral fin rays articulate. Between the superior actinost and the hypercoracoid is a small foramen. A larger foramen occurs between the upper two actinosts. All the others abut solidly (fig, 10).

The pectoral rays (*pc.r.*) are all segmented and they vary greatly in length and thickness. Their number varies from fourteen to seventeen, and, counting from above downward, the seventh or eighth ray is the longest. Above and below this the remaining rays decrease rapidly in length. The lowest ray is the shortest, while the uppermost rays are more slender and delicate. None of the rays are branched. The lower eight are free; the remaining are joined by a delicate membrane (figs. 4 and 10).

The pelvic girdle (*p.g.*) is composed of two long flat bones firmly attached along the middle line. Their dorsal surfaces are grooved near the lateral margins and on the ventral surfaces there are ridges to correspond. At its lower end each bone sends forward a triangular process terminating in a sharp point which unites with its fellow of the opposite side (fig. 4). A wedge-shaped depression between these two processes is noticeable in the unmutilated specimen. The pelvic girdle passes forward and upward between the ventral blades of the clavicles. It is firmly attached to the shoulder-girdle (figs. 4, 10).

The ventral fin rays (*v.f.*) are three in number, segmented and unbranched. The middle ray is one-fourth longer than the outer, and nearly twice as long as the inner ray. There is a very rudimentary spine at the base of each outer ray. The rays are united by a delicate membrane and a single row of papillae (scales) extends along the inner, ventral margin of each ray (figs. 4 and 10).

The posttemporal (*pot.*) is irregularly pyramidal in form and bears on its inner edge a sharp, backward pointing spine. It unites superiorly with the epiotic, anteriorly with the pterotic and opisthotic for a short distance, and inferiorly with the exoccipital. The supraclavicle articulates with the under surface of the posterior spinous process (figs. 4, 5, and 7).

7. *Vertebral column*

Vertebral formula: 12 abdominal + 14 caudal + hypural = 27.

The spine of the atlas is low and the centrum is very short. The zygapophyses, however, are strongly developed, as also are the articular facets of the exoccipitals. The ribs are attached to slightly elevated tubercles high up on the neural processes. On the succeeding vertebrae the rib attachments descend rapidly, and rudimentary transverse processes appear on the fifth. Opposite processes are rather widely separated and are directed ventrolaterally. The transverse processes of the sixth, seventh, and eighth become gradually longer and do not diverge so widely from the median line. The ribs are attached to the distal ends of these processes. No epipleurals are present anterior to the ninth vertebra (fig. 14).

In the remaining abdominal vertebrae the transverse processes unite ventrally to form rather blunt processes which, in the caudal vertebrae, become produced as the haemal spines. The ribs are attached at the lower extremities of these processes and up about one-third of their length are rather prominent elevations to which the large epipleurals articulate. The last three abdominal vertebrae, with their ribs and epipleurals, constitute a characteristic feature of the vertebral column. Figures 15 to 18 illustrate these characters.

The neural spines are strongly developed and are directed somewhat caudally. They increase rapidly in length from the atlas to the fourth vertebra, and then become gradually shorter posteriorly.

The caudal vertebrae (figs. 19 and 20) present no unusual features. The first five or six haemal spines are long and the first three bear intermuscular bones. All are curved in a decidedly caudal direction. The spines are given off from the anterior part of the haemal processes, the latter continuing as delicate lateral walls to the posterior ends of the centra. The last two caudal vertebrae have very strongly developed neural and haemal spines which assist in the support of the hypural. The last three or four vertebrae bear delicate lateral ridges.

The hypural is a fan-shaped bone with a straight caudal edge which has a deep median notch. Six fully developed, segmented, and unbranched caudal rays and one rudimentary ray articulate with the upper part of the hypural; the remaining five rays are fully developed and articulate with the lower part. In the average adult specimen the rays are about 9 to 14 mm. long, the inferior rays being the shortest and the middle ones the longest. The uppermost ray bears a row of spines on its dorsal margin (fig. 14).

The interneural spines decrease gradually in length from before backward. They have prominent lateral ridges, are wedge-shaped ventrally, and have large backward-pointing heads for articulation with each other and with the dorsal spines and rays. The first interneural spine is very large and probably represents the ankylosed first two since it supports the first two dorsal spines (fig. 14).

The dorsal spines, eight in number, are weak and slender. The last one is very short (about 1.3 mm.) and may easily be overlooked. The longest are 5 to 7 mm. and the first bears delicate spines on its anterior edge. The proximal ends of the spines are expanded into large, rounded heads which articulate in sockets or grooves of the intesneurals (fig. 14).

The rays of the second dorsal are much more strongly developed, ranging from 7 to 10 mm. in length. The first has spines on its anterior edge, and most of the others have delicate spines on their sides (fig. 14).

Except for being a little more slender, the interhaemal spines do not differ from the interneurals. There are six, and the last one bears the last two anal rays. The superior end of the first lies between the lower ends of the first two haemal spines. The last one is exceedingly short and slender.

The anal rays are like the dorsal, segmented and unbranched. They range in length from 6 to 12 mm., the longest being in the middle. Seven is the usual number, although specimens were observed which had but six.

III. DISCUSSION AND CONCLUSION

From the foregoing it is evident that the families Cottidae and Rhamphocottidae are very closely related so far as osteological characters are concerned. This fact is emphasized by categorical comparison of the two forms. The differences, however, appear to be of sufficient value to elevate Rhamphocottus to the rank of a separate family, although opinion in this regard may differ, as there is wanting, unfortunately, a universal standardization of taxonomic values assigned to given morphological characters by different authors. Not infrequently the differences are only differences of degree, and this appears to be the situation in the case of Rhamphocottus. Nearly all of the characters assigned to distinguish the Rhamphocottidae may be found represented in a greater or lesser degree in some member of the Cottidae. The length of the body, for example, is subject to great variation in the Cottidae. It may be short (Enophrys bison, Ceratocottus dicerans) or greatly elongated, as in Triglops and Radulinus. The body of Histiocottus bilobus is short, deep, and compressed, the interorbital space is concave, and occipital ridges are developed. In no case, however, are these characters so exaggerated or so prominent as they are in Rhamphocottus.

In Rhamphocottus there is no slit behind the last gill, but this can hardly be regarded as a family character in this instance, for a similar condition is found in a number of the Cottidae. The condition of the gill membranes is also too variable to be of any special significance.

The number of vertebrae is slightly less in Rhamphocottus than in the Cottidae. The vertebrae were counted in seven skeletons, and in each the number was twenty-seven, counting the hypural. Jordan and Evermann give the number as twenty-four in Rhamphocottus and thirty to fifty in the Cottidae.

The myodome is present in both families (Jordan and Evermann state the contrary for Rhamphocottus), and the number of pyloric caeca in Rhamphocottus (five) falls within the limits found in the Cottidae.

The osteological characters of Rhamphocottus which stand out most prominently are the great length of the snout, the

marked concavity of the superior surface of the cranium, the short, elevated, and compressed form of the skeleton as a whole, and the smooth fibrous nature of the large bony ridges.

In addition to osteological differences, there are certain superficial characters which are more or less striking. Of these may be mentioned the rich color pattern and the unusual type of scales.

While it does not appear that Rhamphocottus diverges enough from the Cottidae to justify the creation of a superfamily, the differences are, in my opinion, of family value. Such an unusual combination of striking characters is certainly not found in any typical member of the Cottidae. This conclusion is in accord with the classification of Jordan and Starks and of Jordan and Evermann, but differs from the opinion expressed by Gill that Rhamphocottus probably represents a superfamily and from the original classification of Günther who placed Rhamphocottus with the Cottidae.

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ABBREVIATIONS

ac., actinost
ab.v., abdominal vertebra
a.f., anal fin
als., alisphenoid
an., angular
ar., articular
a. zyg., anterior zygapophysis
bbr., basibranchials
bo., basioccipital
br., branchiostegal rays
c., centrum
cbr., ceratobranchials
c.f., caudal fin
chy., ceratohyal
cl., clavicle
d., dentary
d.r., dorsal rays
d.s., dorsal spines
e., ethmoid
epbr., epibranchials
eo., exoccipital
ephy., epihyal
epo., epiotic
eppl., epipleural
fr., frontal
h., hypural
hbr., hypobranchials
hhy., hypohyals
hl. pr., haemal process
hm., hyomandibular
hl. sp., haemal spine
hpc., hypercoracoid
hpoc., hypocoracoid
hy., hyoidean arch
in. sp., interneural spine
ihy., interhyal
ih. sp., interhaemal spine
iop., interoperculum
i.ph., inferior pharyngeal
m., maxilla
mc., mesocoracoid
mpt., metapterygoid
mspt., mesopterygoid
na., nasal
n. pr., neural process
n. sp., neural spine
op., operculum
opo., opisthotic
p., parietal
pa., palatine
pas., parasphenoid
pcl., postclavicle
pf., prefrontal
pc. r., pectoral rays
p.g., pelvic girdle
pm., premaxilla
pop., preoperculum
por., preorbital
pot., posttemporal
pro., prootic
pto., pterotic
ptor., postorbital
pt., pterygoid
p. zyg., posterior zygapophysis
q., quadrate
r., rib
scl., supraclavicle
so., supraoccipital
s.g., shoulder-girdle
s. ph., superior pharyngeal
sor., suborbital
spo., sphenotic
sy., symplectic
v., vomer
v.f., ventral fin

PLATE 1

EXPLANATION OF FIGURES

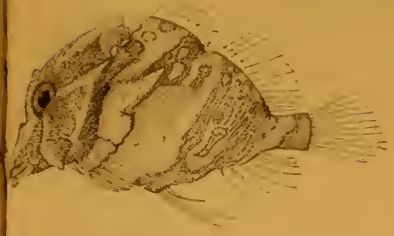
1 Rhamphocottus richardsoni Günther, natural size.

2 A small piece of integument magnified to show the character of the scales and papillae. × 30.

3 Section through a single papilla, showing the scale extending as a rod-like process from a bony plate beneath the epidermis and dividing near its tip to form three prickles. × 90.

4 Lateral view of entire skeleton. × $2\frac{1}{2}$.

5 Lateral view of the cranium. × 4.



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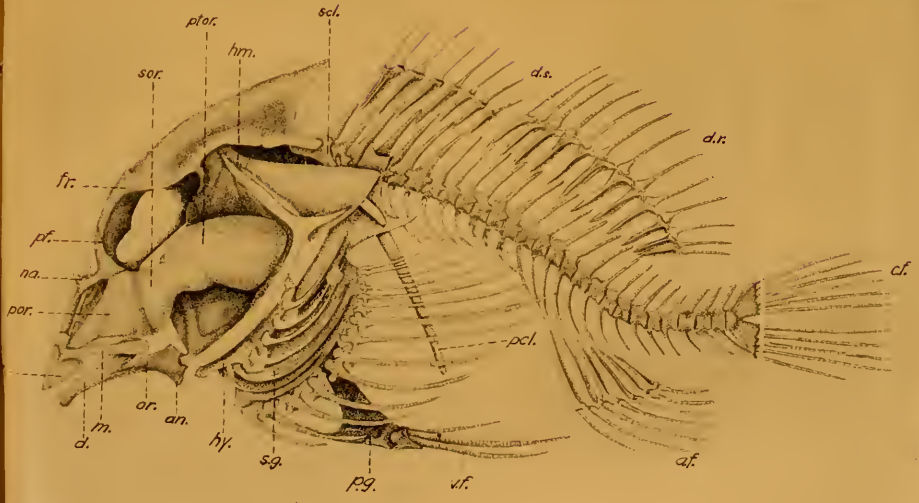
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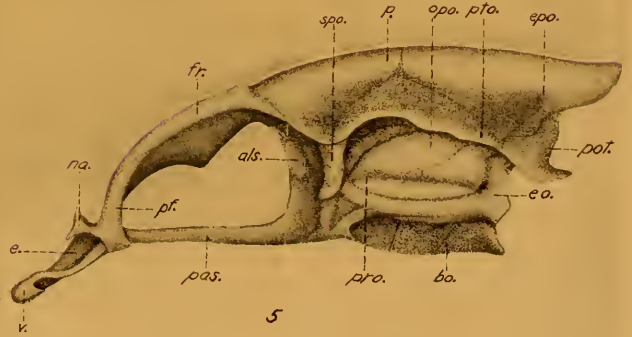
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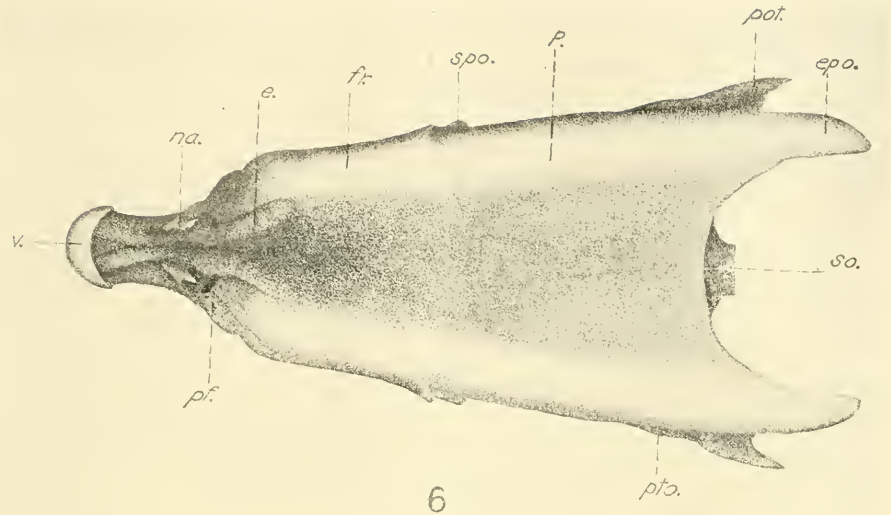
PLATE 2

EXPLANATION OF FIGURES

6 Dorsal view of cranium. × 4.
7 Ventral view of the cranium. × 4.
8 Posterior view of the cranium. × 4.
9 Maxillary and premaxillary bones. × 5.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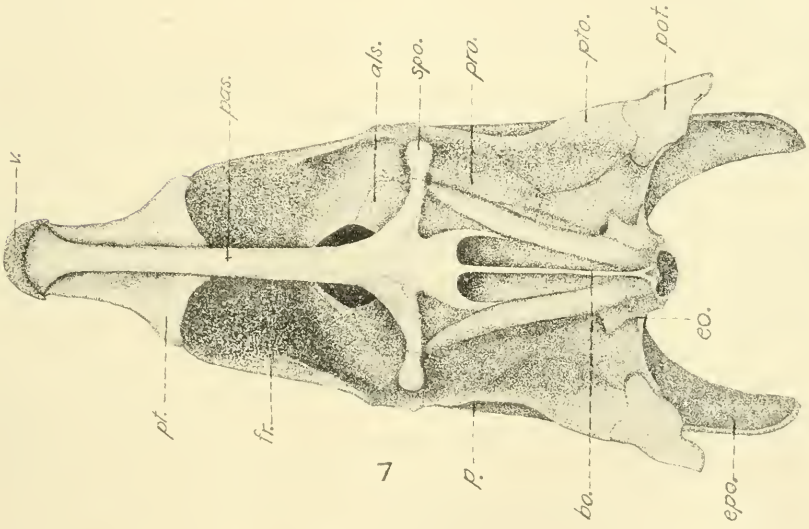
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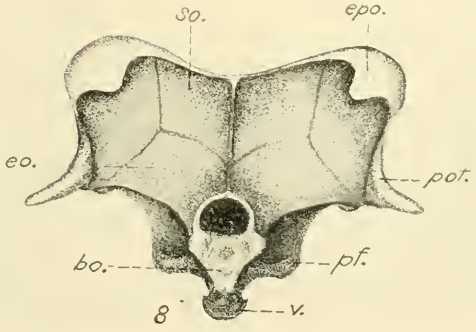
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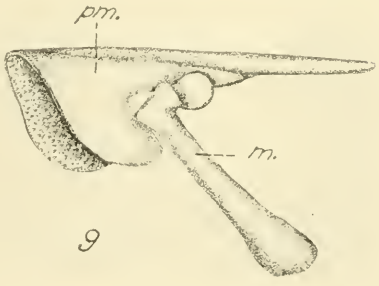
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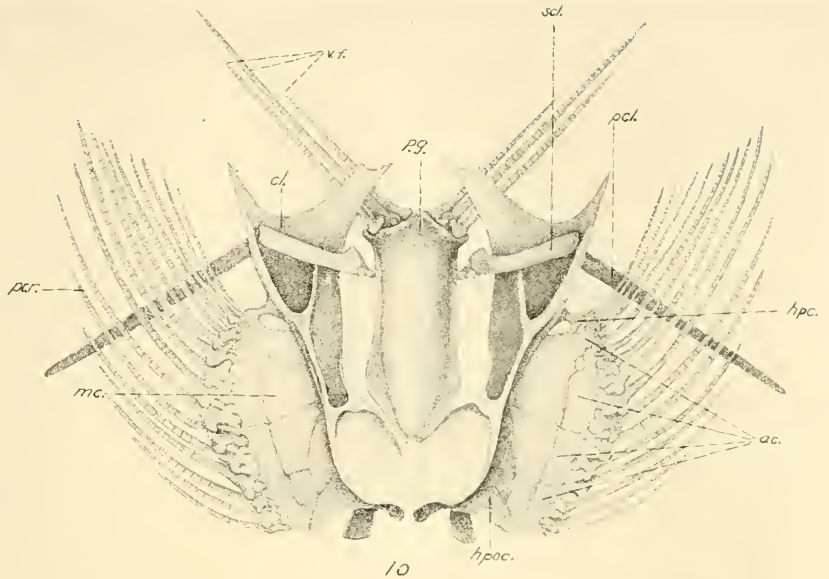
PLATE 3

EXPLANATION OF FIGURES

10 Shoulder and pelvic girdles. Posterodorsal view. × $2\frac{2}{3}$.
11 Hyoidean apparatus. × 4.
12 Branchial arches. × 4.
13 Suspensorium and opercular apparatus. × $2\frac{2}{3}$.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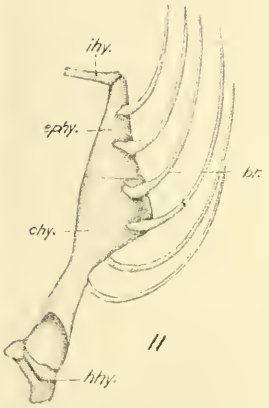
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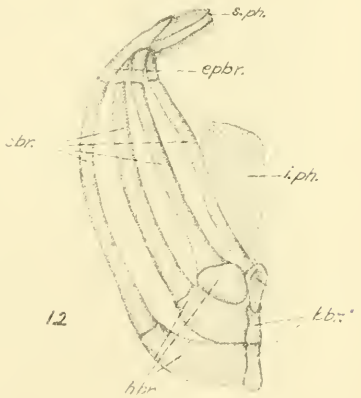
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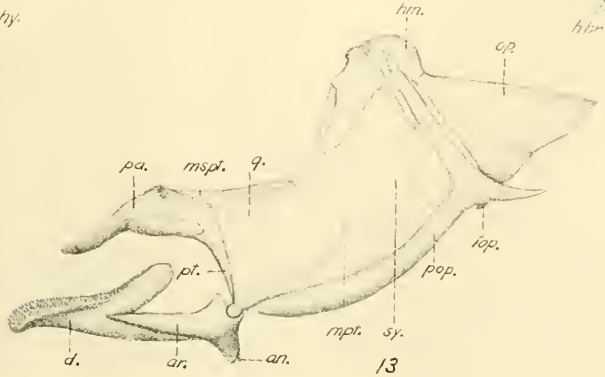
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d.
ar.
an.

13

PLATE 4

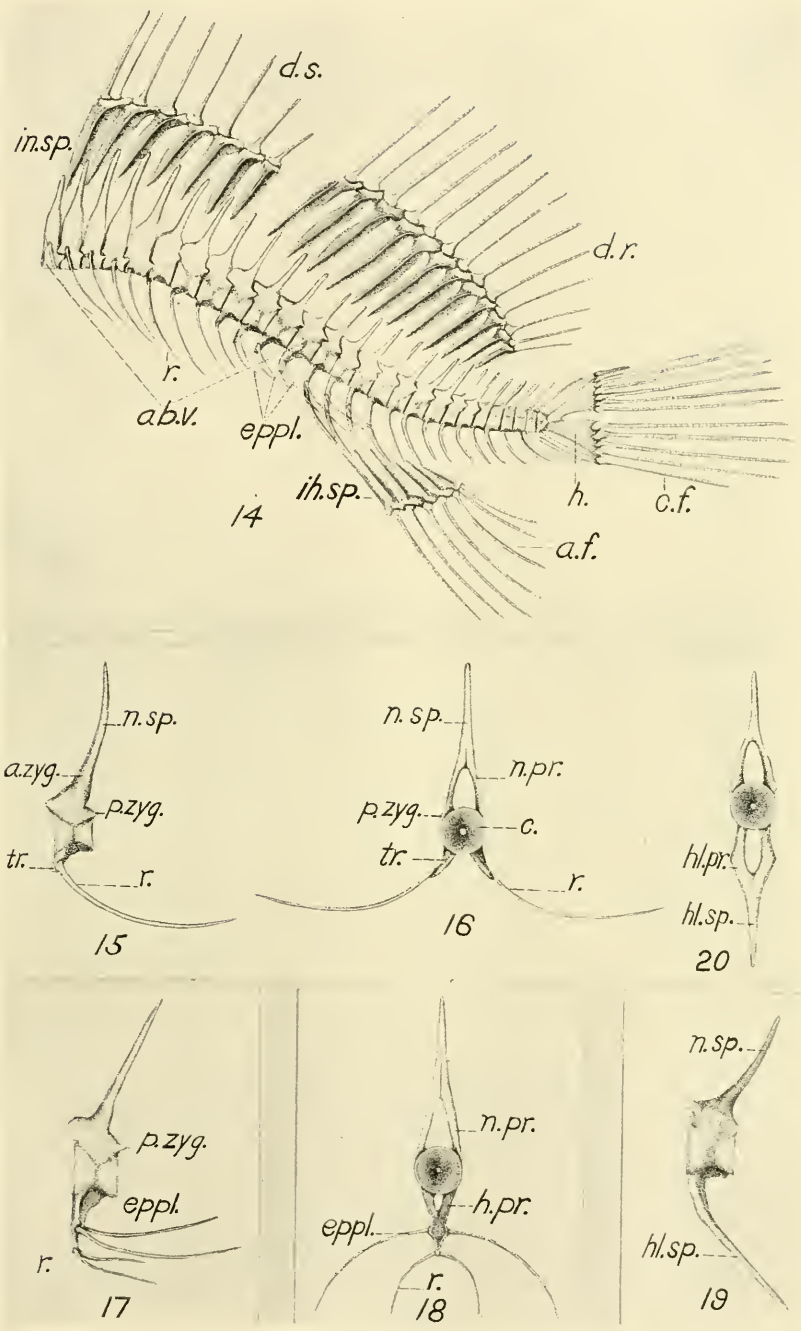
EXPLANATION OF FIGURES

14 Lateral view of the vertebral column. × $1\frac{1}{2}$.

15 and 16 Lateral and posterior views of the eighth abdominal vertebra. × $2\frac{1}{2}$.

17 and 18 Lateral and posterior views of the tenth abdominal vertebra. × $2\frac{1}{2}$.

19 and 20 Lateral and posterior views of a caudal vertebra. × $2\frac{1}{2}$.


d.s.
in.sp.
d.r.
r.
a.b.v.
eppl.
ih.sp.
h.
c.f.
a.f.
14
n.sp.
a.zyg.
p.zyg.
tr.
r.
15
n.sp.
n.pr.
p.zyg.
c.
tr.
r.
16
hl.pr.
hl.sp.
20
p.zyg.
eppl.
r.
17
n.pr.
h.pr.
eppl.
r.
18
n.sp.
hl.sp.
19

Resumido por los autores H. W. Norris y Sally P. Hughes.

Los nervios craneales y espinales anteriores de los anfibios cecílidos.

El nervio olfatorio aunque es doble es simplemente una exageración del tipo normal de los anfibios. La estructura rudimentaria del nervio óptico y la de los nervios de los músculos oculares presenta dos grados: a) en uno de ellos existen todos, pero a excepción del abductor, muy reducidos; b) en el otro faltan todos a excepción del abductor. La división oftálmica del nervio trigémino está separada de la maxilo-mandibular. No existe anastomosis entre el ramo oftálmico profundo del nervio V y el ramo palatino del VII, pero hay una anastomosis entre este último y el ramo maxilar del V. Existe un elemento de la linea lateral en el facial del adulto, asi como un contingente lateral completo en el facial y vago de las larvas. El ramo yugular del VII está extraordinariamente desarrollado, en relación con la considerable extension posterior de los músculos del arco hioideo. Los ganglios del vago y glosofaríngeo están generalmente bien manifiestos; el del primero es doble. Existe un nervio occipital. El nervio hipogloso nace del primer nervio espinal o de este y el occipital. El tronco del simpático está relacionado anteriormente con los ganglios de los nervios V y VII. Dos ganglios del simpático (raras veces más) están en relación con los nervios espinales, en parte por anastomosis y en parte por simple aproximación. Los siguientes nervios craneales característicos de los anfibios faltan en los cecílidos: a) la comisura de Jacobson; b) el "ramo comunicante" entre los nervios IX a X y VII; c) el ramo pretrématico del IX; d) el ramo auricular del X. Las formas estudiadas son las siguientes: Dermophis, Ichthyophis, Geotrypetes, Herpele y Caecilia.

Translation by Dr. José Nonidez,
Columbia University

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THE CRANIAL AND ANTERIOR SPINAL NERVES OF THE CAECILIAN AMPHIBIANS

H. W. NORRIS AND SALLY P. HUGHES

The Zoological Laboratory of Grinnell College

FORTY-FOUR FIGURES

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INTRODUCTION

The earliest account of the nervous system of the caecilians seems to be that of Fischer ('43), describing that of Caecilia (Siphonops) annulata. His account of the fifth, seventh, ninth, tenth, and twelfth nerves is more nearly correct than that of some later writers. He considers the caecilian cranial nerves arranged on the general amphibian plan despite the external differences between caecilians and other amphibians. He recognizes a well-defined sympathetic trunk arising anteriorly from both the fifth and the seventh nerves. He describes and figures an intimate association between the sympathetic trunk and the glossopharyngeal, vagus, and anterior spinal nerves. The lateral line nerve of the trunk he considers derived from the third spinal nerve.

Wiedersheim ('79) describes the cranial nerves of Epicrium (Ichthyophis) glutinosum and Siphonops annulatus in general agreement with the account of Fischer. The double nature of the olfactory nerve he homologizes with that of a spinal nerve. The optic nerve he finds in a vestigial condition, but fails to trace it to the eyeball. Of the eye-muscle nerves he makes no mention. The double nature of the fifth nerve and the origin of the ramus ophthalmicus (r. oph. profundus) independent of the Gasserian ganglion are recognized. He describes a supposed anastomosis between the ramus ophthalmicus and the ramus maxillaris. The masseter muscle and the compressor muscle of the orbital gland he believes innervated by the ramus maxillaris. In the facial nerve he finds no ramus palatinus, but believes the latter to be incorporated in the trigeminus. From the facial nerve he finds an anastomosis entering the Gasserian ganglion. The acusticus is a well-defined nerve. In the glossopharyngeal-vagus group he describes and figures three nerves: an anterior glossopharyngeal with the usual distribution, a middle vagus, and a posterior nerve entering the sympathetic trunk. Anastomoses of the ninth and tenth nerves with the sympathetic are also described. A ramus lateralis of the vagus he does not find. The hypoglossus is formed from the first and second

spinal nerves, both of which anastomose with the sympathetic trunk. The sympathetic chain begins anteriorly with branches from the Gasserian and facialis ganglia. In the region of the first spinal nerve is developed a large cervical sympathetic ganglion, with which a large branch of the vagus anastomoses. The first spinal nerve enters this cervical ganglion and on emerging fuses with a branch of the second spinal nerve to form the hypoglossal nerve. Three other sympathetic ganglia posterior to the cervical are figured. With the sympathetic cord he believes the spinal nerves anastomose.

Retzius ('81) is unable to find an auditory nerve in Caecilia (Siphonops) annulata, unless certain rudimentary tube-like structures represent it.

Waldschmidt ('87) rejects Wiedersheim's assumption of the spinal nerve homology of the olfactorius in the caecilians. In Siphonops annulatus he finds an oculomotorius which he believes to anastomose with the ramus maxillaris V. The rest of the oculomotor nerve he thinks supplies the compressor muscle of the orbital gland, one of its branches combining with a small branch of the ramus maxillaris V. He fails to find a trochlearis or an abducens nerve. He agrees with Wiedersheim as to the general structure of the trigeminus, but finds a fine cutaneous nerve arising apparently from the Gasserian ganglion, which, on the whole, seems to him to correspond to a lateral line nerve (ramus ophthalmicus superficialis VII) of larval amphibians and fishes. He finds what he considers the ramus palatinus VII. Of the existence of a functional acusticus he is not certain. The ganglion of the vagus nerve fuses with a sympathetic ganglion, and the vagus trunk sends anastomoses to the sympathetic chain and to the hypoglossus.

The Sarasins ('90) find the auditory nerve characteristically developed in Ichthyophis glutinosus. The ventral division of the olfactorius supplies Jacobson's organ.

Burckhardt ('91) compares the olfactorius of Ichthyophis with that of the Urodela in general and finds no fundamental differences, the double nature in the caecilians being but an exaggeration of the general amphibian relationships of the olfactorius.

Like Wiedersheim and Waldschmidt, he fails to find a trochlearis or an abducens. He recognizes four roots in the acusticus, three roots in the glossopharyngeus, and seven roots in the vagus. He finds two distinct divisions in the IX to Xth ganglion of the embryo: an anterior small-celled portion and a posterior part with large cells. From the vagus ganglion he derives a cervical sympathetic ganglion, which remains connected with the vagus by a large nerve trunk. The double nature and origin of the fifth nerve is shown by figures of the embryo of Ichthyophis.

According to Brauer ('04), the double condition of the fifth nerve in Hypogeophis is related to the distinct origins of the two parts in the embryo, the ophthalmic ganglion coming from a dorsolateral placode, and the maxillomandibular ganglion from the neural crest.

Marcus ('10) has given us a thoroughgoing account of the structure of the caecilan head and the homologies of the organs therein represented. An examination of his figures shows that the cranial nerves of a caecilian are fundamentally like those of other Amphibia. He finds all three eye-muscle nerves represented in the embryo, with the characteristic distribution. The sixth nerve, besides supplying the rectus lateralis muscle, innervates the retractor tentaculi, the latter probably a retractor bulbi in origin. The fourth nerve is very small, but of the eye-muscle nerves the third nerve is the largest. Besides its characteristic distribution, he believes that it supplies the levator bulbi muscle. He agrees with Brauer as to the origin of the fifth nerve. It is evident that the facialis contains in the embryo a complete lateral line component: rami ophthalmicus superficialis, buccalis, and probably mandibularis externus. A ramus recurrens VII passes posteriorly around the ear capsule to unite, not with the ninth or tenth nerves, but with the great cervical sympathetic ganglion. In the IX to X complex he finds eight to ten roots. The ganglion in the embryo is plainly double, an anterior ninth and a posterior tenth ganglion. In one stage he figures the tenth ganglion as double. The ninth nerve (first branchial) shows a division into pre- and post-trematic branches. The second, third, and fourth branchial nerve divisions of the

vagus are not complete, showing apparently only post-trematic elements. The main part of the tenth nerve is directed posteriorly, but divides into an anterior recurrent branch [ramus laryngeus recurrens] and a posterior division [intestinalis]. Of the lateral line nerves of the trunk he finds four representatives running superficially: a dorsal lateralis superior, two laterales mediales, and a ventral lateralis inferior. These he homologizes with the lateral line nerves in the salamander. Arising from the third spinal nerve and extending posteriorly is a nerve which he terms lateralis profundus. He derives the hypobranchial (hypoglossal) from the first and second spinal nerves. He finds a spino-occipital present. The sympathetic ganglia come, not from the spinal ganglia, but from the neural crest. He figures two sympathetic ganglia: an anterior cervical near the posterior vagus ganglion and a second farther posteriorly at a level between the third and fourth spinal nerves.

The contributions of Luther ('14) to a clarification of homologies in amphibian anatomy have only an indirect bearing upon a consideration of the cranial nerves in caecilians, but the writers must acknowledge great indebtedness to this author for his convincing statements regarding the homologies of the muscles innervated by the fifth nerve in the caecilians.

MATERIAL AND METHODS

This paper is based upon the study of adults of Herpele ochrocephalum, collected for the writers in Ancon, Panama. The material was fixed and preserved in 10 per cent formalin. Subsequently when it came into the hands of the writers it was treated with the vom Rath picro-acetic-osmic-platinic mixture in the way usually employed for fresh material. Serial sections of the head were prepared by the celloidin method.

To the kindness and generosity of Dr. S. T. Darling, of the Canal Zone Board of Health Laboratory, more recently of the International Health Commission, the writers are indebted for the specimens of Herpele. As specimens of this form had been sent by Dr. Darling to Dr. L. Stejneger, of the National Museum, for identification, it may be assumed that the specific name is

correctly employed. Dr. A. G. Ruthven, of the University of Michigan, collected and fixed by the vom Rath method a specimen of Dermophis mexicanus in southern Mexico. A specimen of Caecilia gracilis, collected by him in British Guiana, was also contributed. Thanks are due Dr. Thomas Barbour, of the Museum of Comparative Zoology, Harvard University, for his fruitful suggestions and the gift of specimens of Geotrypetes petersii from German West Africa, and Ichthyophis beddomii (glutinosus?) from India. Prof. J. S. Kingsley, of the University of Illinois, kindly loaned his serial sections of heads of larval Ichthyophis glutinosus. To the trustees of the Bache Fund of the National Academy of Sciences acknowledgment is made of a grant that greatly expedited the completion of this research.

Two types of cranial structure are found in the caecilians: 1) eye covered or nearly covered by the maxilla, eyeball very rudimentary, no optic nerve, no eye-muscle nerves except the abducens, no eye-muscles (Caecilia, Herpele) (fig. 10); 2) eye not covered by the maxilla, but shows characteristic structure with muscles and nerves (Ichthyophis, Dermophis, Geotrypetes) (figs. 11 to 13, 17, 19). In the second group there is a peculiar lateral shelf-like extension of the ethmoid bone, internal septum (processus conchoides of the Sarasins), which bears near its free border the tubular passageway of the ventral olfactory nerve (figs. 6, 7, 11, 12). In Herpele and Caecilia, and apparently in Hypogeophis (Wiedersheim), the ventral olfactory nerve runs in a canal in the base of the internal septum (fig. 8, *Iv.*). The development of a processus conchoides produces other modifications of the nasal topography. Jacobson's organ, which, in Herpele and Caecilia (figs. 8, 9, *jo.*), connects with the mesial portion of the nasal chamber, in the other type communicates with the lateral portion (figs. 6, 7, *jo.*).

ABBREVIATIONS

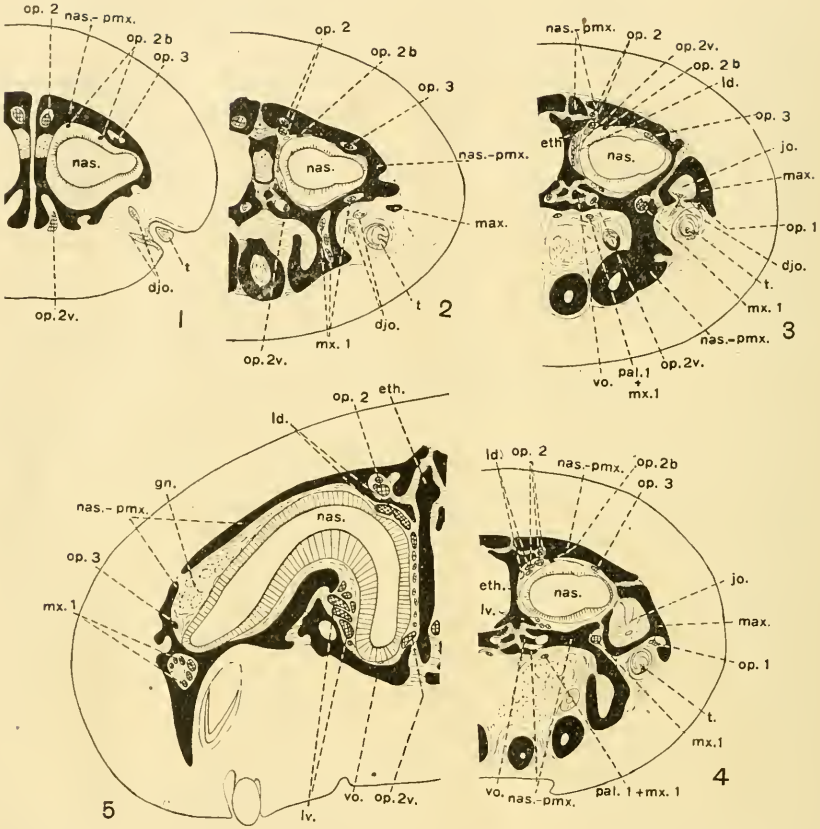
atv., ramus alveolaris VII.
alv. +*md,4a*, union of alveolar branches of r.alv.VII and r. mandibularis V.
anast.IX–X, fibrous connection, probably sympathetic, between the glossopharyngeal and vagal ganglia in Dermophis.
anast.IX-sy., a nerve passing from the glossopharyngeal trunk into the first cervical sympathetic ganglion.
anast.sp.3+4-gsy.2, fibrous connections between the third and fourth spinal nerves and the second cervical sympathetic ganglion in Ichthyophis.
ar., arytenoid cartilages.
auc., ear capsule
ba., os basale
br., brain
br.1,br.2 etc., branchial arches
buc., r. buccalis VII
buc.1, lateral division of r. buccalis VII in larval Ichthyophis
buc.2, mesial division of r. buccalis VII in larval Ichthyophis
c.1,c.2, etc., ribs
cal., m. constrictor aditus laryngis
cgo., m. compressor glandulae orbitalis
ch., cerebral hemisphere
chb., "Choanabucht," a pouch opening into the choana
che., m. ceratohyoideus externus
chi., m. ceratohyoideus internus
cho., choana
cl.2, second branchial cleft
csp., chorda spinalis
de., diencephalon
dent., os dentale
djo., duct of Jacobson's organ
dla., m. dorsolaryngeus (a) = hyopharyngeus
dlb., m. dorsolaryngeus (b) = hyopharyngeus internus
dlc., m. dorsolaryngeus (c) = dilatator laryngis
dm., m. depressor mandibulae and branches of r. jugularis VII supplying it.
dma., anterior division of m. dep. mand., and branch of r. jugularis VII supplying it
dmp., posterior division of m. dep. mand. and branch of r. jugularis VII supplying it.
eth., os ethmoideum
fr., os frontale
gac., ganglion acusticum
gacs., ganglion acusticum, saccular portion
gen., ganglion geniculi
gg., ganglion gasseri
ggl., ganglion glossopharyngeum
gh., m. geniohyoideus
gj., glandula Jacobsoni
glld., ganglion lineae lateralis dorsale facialis
gllv., ganglion lineae lateralis ventrale facialis
gn., glandula nasalis
go., glandula orbitalis
gon.,os goniale = gonio-articulare
gop., ganglion ophthalmicum profundum
gor., glandulae oris
gpal., ganglion palatinum
gsp.2,gsp.3. etc., spinal ganglia
gsy.1,2, first and second cervical sympathetic ganglia
g.IX., ganglion glossopharyngeum
g.Xr., root ganglion of vagus nerve
g.Xtr., trunk ganglion of vagus nerve
hgl., n. hypoglossus
hthl., hypothalamus
hy., hyoid cartilage
hygl., m. hyoglossus
ib.4, m. interbranchialis 4
ih., m, interhyoideus
im., m. intermandibularis
int., r., intestinalis X
int.-acc., r. intestino-accessorius X, only in part corresponding to the nerve of that name in the Urodela
io., m. obliquus ventralis
itr., mm. inter-transversales
jgl., r. jugularis VII

jo., organon Jacobsoni
lab., m. levator arcuum branchialium
lar., larynx
lar.rec., r. laryngeus recurrens X
lbaur., "lobus auricularis" of med. obl.
lbll., lobus lineae lateralis
lbr., m. levator branchiae
ld., mm. latissimi dorsi
lh., m. levator hyoidei
lq., m. levator quadrati
mas.1., m. masseter anterior, main portion of masseter muscle
mas.2, m. masseter posterior = adductor mandibulae minor of Luther
max., os maxillare
maxp., palatine process of maxilla
mck., cartilago Meckelii
md., r. mandibularis V
md.1., small ramulus of r. md. V innervating the masseter, temporal and compressor of orbital gland muscles
md.2, lateral sensory ramulus of r. md. V
md.4, rm. mandibularis externus X
md.4a, rm. alveolaris of rm. md. ext. V.
md.5, rm. intermandibularis V
mo., medulla oblongata
mtl.ext., r. mentalis externus VII
mtl.int., r. mentalis internus VII
mx., r. maxillaris V
mx.1, medial ramulus of r. mx. V
mx.1 + *pal.2*, temporary fusion of branches of medial ramulus of r. mx. V and lateral ramulus of r. palatinus VII
mx.2, lateral ramulus of r. mx. V
nas., nasal cavity
nas.-pmx., os naso-premaxillare
nc., cartilago nasalis
oc., eyeball
occ., condylus occipitalis
ocn., n. occipitalis
op., r. ophthalmicus profundus V
op.1, rm. ophthalmicus profundus minor (in part)
op.1 + *os.*, union of r. oph. superficialis VII with rm. op. 1, V
op.2, rm. nasalis internus = main trunk of r. oph. prof. V
op.2b, small branches of r. oph. prof. V innervating olfactory membrane
op.2v, ventral ramulus of rm. nas. int. supplying the skin on ventral surface of snout
op.3, rm. nasalis externus
os., r. ophthalmicus superficialis VII
osph., orbitosphenoid bone or cartilage
pa., os parietale
pal., r. palatinus VII
pal.1, mesial ramulus of r. pal. VII
pal.1 + *mx.1*, union of rm. pal. 1 with a branch of the mesial ramulus of r. mx. V
pal.2, lateral ramulus of r. pal. VII
pl., os palatinum
psph., os. parasphenoidale
pt., m. pterygoideus and nerve supplying it
ptc., cartilaginous articulation between the pterygo-quadrate and basal bones
ptfr., os postfrontale
pt.-qu., os pterygo-quadratum = suspensorium
ra., m. rectus abdominis
ral., m. rectus abdominis lateralis
rath., m. rectus abdominis thoracico-hyoideus
rext., m. rectus lateralis
rinf., m. rectus ventralis
rint., m. rectus medialis
rs., m. rectus dorsalis
rsv., m. rectus subvertebralis
rt., m. retractor tentaculi
rtb., m. retractor bulbi
rts., retractor muscle of the tentacular sheath
sa., mm. subarcuales
sd., muscle designated as "Seitswartskrummer des Rumpfes" by Wiedersheim
ser., m. serratus (of Wiedersheim)
so., m. obliquus dorsalis
sphc., equivalent of m. sphincter colli of the Urodela
sp.V, tractus spinalis trigemini
sp.1, *2*, etc., spinal nerves
sp.1d., *2d.*, etc., dorsal rami of spinal nerves

sp.1v., 2v., etc., ventral rami of spinal nerves
sp.2r., 3r., etc., roots of spinal nerves
sp.2vc., 3vc., etc., cutaneous ramuli of the ventral rami of spinal nerves
sp.2vm., 3vm., etc., motor ramuli of the ventral rami of spinal nerves
sp.2vmsv., 3vmsv., etc., motor ramuli of the ventral rami of spinal nerves, innervating the rectus subvertebralis musculature
sp.2l.,3l., etc., lateral rami of spinal nerves
sp.3vcl., 4vcl., etc., lateral cutaneous ramuli of the ventral rami of spinal nerves
sy., sympathetic trunk
sy.V, origin of the sympathetic trunk from the Gasserian ganglion
sy.VII, origin of the sympathetic trunk from the geniculate ganglion
t., tentacle
th., thymus
thr., glandula thyreoidea
tm., m. temporalis
tmpro.. caput preorbitale of m. temporalis
tr., trachea
tr.b., 'tract b' of Kingsbury
trc., trabecular cartilage
vo., vomer
v.1, 2, etc., vertebrae
I., n. olfactorius
Id., dorsal division of n. olf.
Iv., ventral division of n. olf.
II., n. opticus
III, n. oculomotorius
IV., n. trochlearis
V., n. trigeminus
Vd., 'dorsal V'
Vrmd-mx., root of the maxillo-mandibular division of the trigeminal nerve
Vrop., root of the ophthalmicus profundus division of the trigeminal nerve
VI, n. abducens
VII, n. facialis
VIIr., radices facialis
VIIrc., radix communis facialis
VIIrll., radix lineae lateralis facialis
VIIrm., radix motor facialis
VIII, n. acusticus
VIIIa., radix anterior of n. acusticus
VIIIp., radix posterior of n. acusticus
VIIIr., radices acustici
IX, n. glossopharyngeus = n. branchialis primus
IXph., r. pharyngeus IX
IXphl., r. pharyngeus lateralis IX
IXpst., r. posttrematicus IX
IXr., radix glossopharyngei
IX–X, common trunk of ninth and tenth nerves
X, n. vagus
Xlat., r. lateralis X
Xr., radices vagi
X.1, n. branchialis secundus
X.1ph., r. pharyngeus of the second branchial nerve
X.1php., posterior pharyngeal branch of the second branchial nerve
X.1pst., r. posttrematicus of the second branchial nerve

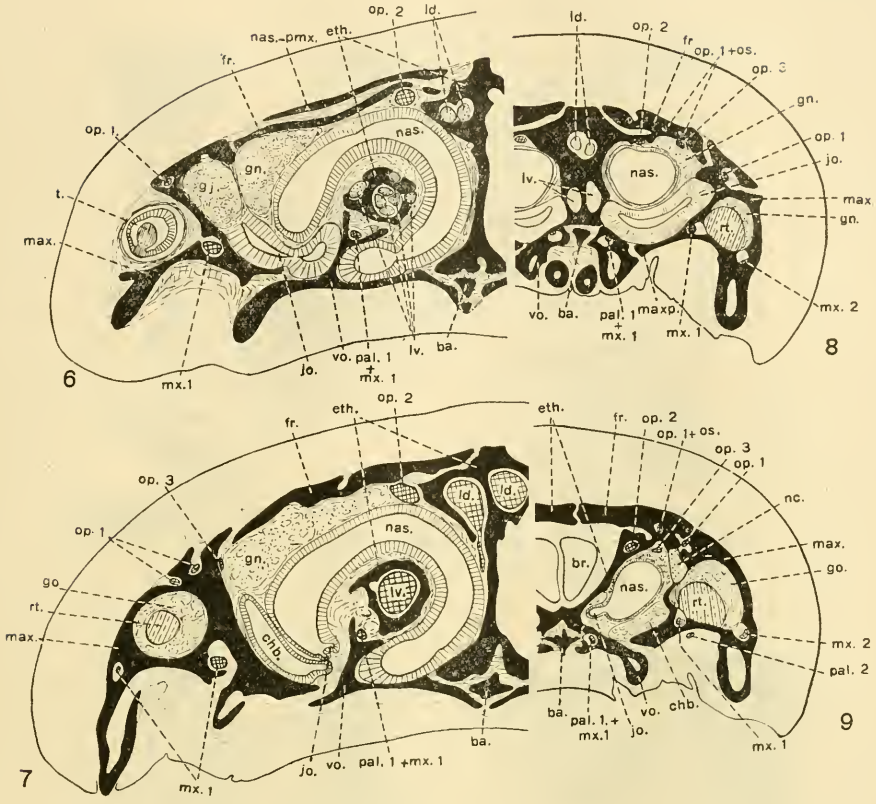
THE OLFACTORY NERVE

As noted above, Burckhardt considers the double condition of the olfactorius in Ichthyophis as more apparent than real. According to him (l.c., p. 392), each of the two parts of the olfactory nerve, dorsal and ventral, on entering the olfactory bulb, sends bundles of fibers into the other, forming in a way a chiasma between the two divisions. It is evident in Herpele, Dermophis,



Figs. 1 to 4 Cross-sections through the head, left half, upper jaw, of Herpele ochrocephalum; anterior nasal region; sections 52, 66, 74 and 85 in fig. 44. × 12.5.

and Geotrypetes that, on the entrance of the ventral olfactorius into the brain, bundles of fibers ascend into the dorsal glomeruli. That the dorsal division sends fibers into the ventral glomeruli is not so clear, but apparently this occurs in Herpele. The arrangement of the olfactory glomeruli in the caecilians is in two groups, not, however, dorsal and ventral as Burckhardt assumes, but in a posterior lateral group and an anterior medial and lateral group. From the former arise the fibers that supply Jacobson's organ. These latter, with fibers from the anterior glomeruli,

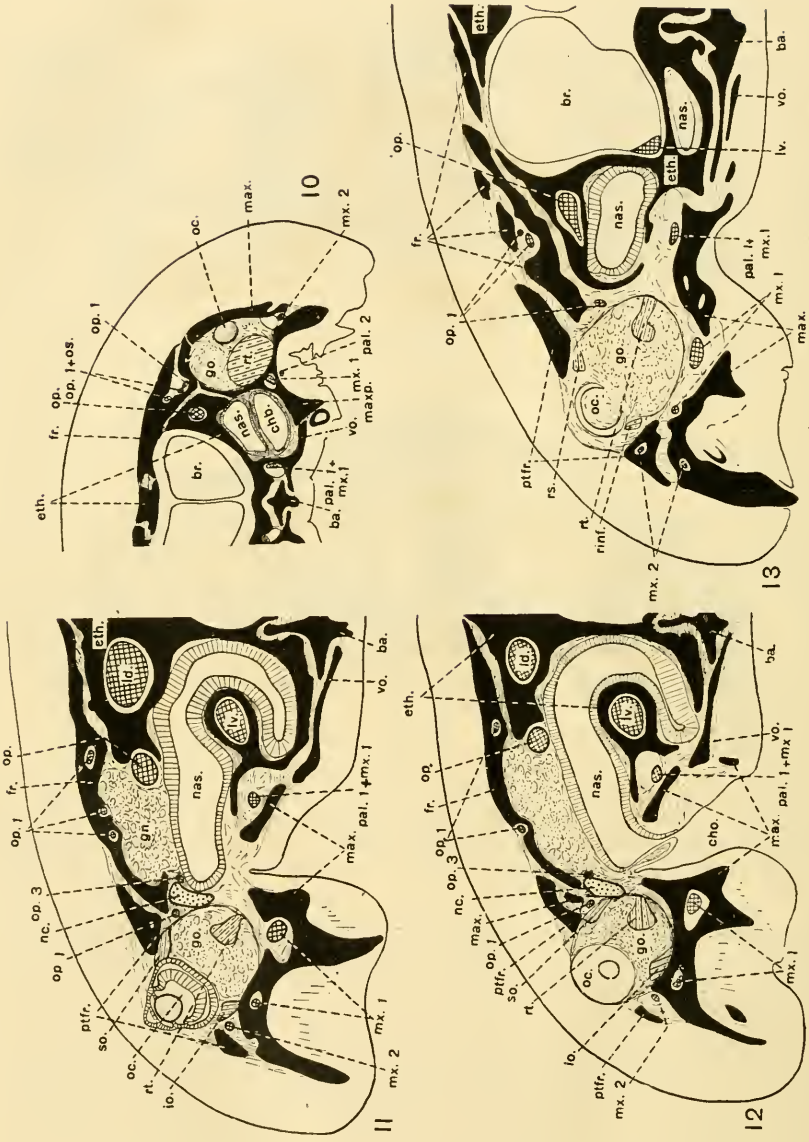


Figs. 5 to 7 Cross-sections through the head, right half, upper jaw, of Dermophis mexicanus; middle and posterior nasal region. × 12.5.

Figs. 8 to 10 Cross-sections through the posterior nasal region of Herpele; fig. 10 through the eyeball; sections 115, 127 and 135. × 12.5.

constitute the ventral division of the olfactory nerve. The dorsal division of the nerve arises from the anterior glomeruli, both medial and lateral.

Although, as shown by Burckhardt ('91), Lee ('93), and Norris ('08, '13), the olfactory centers are double in the Urodela, peripherally there is no such sharp distinction. It would seem from the condition in Amphiuma and Siren (Norris, '08, '13) that Jacobson's organ is related to the ventral posterior glomeruli, but that with the latter are also connected portions of the defini-


10
11
12
13
eth.
fr.
op.
op. 1+os.
op. 1
oc.
max.
mx. 2
go.
rt.
nas.
chb.
br.
vo.
maxp.
mx. 1
pal. 2
pal. 1+
mx. 1
ba.
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nc.
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pal. 1+mx. 1
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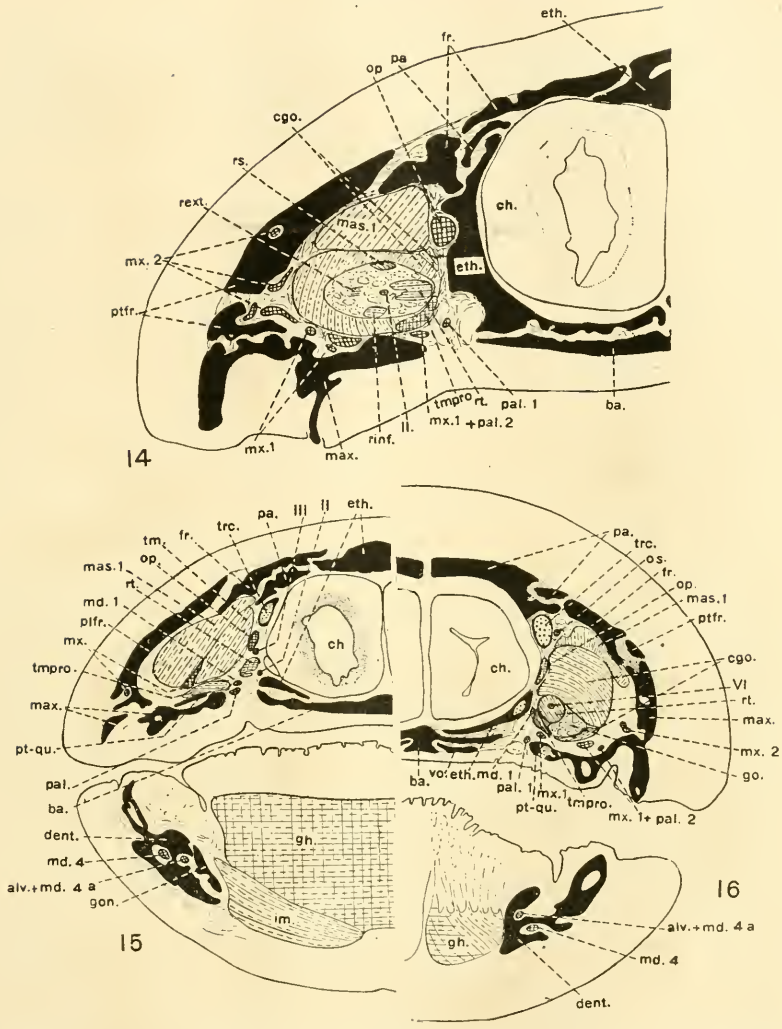


Figs. 11 to 14 Cross-sections through the head, right half, of Dermophis; figs. 11 to 13 through the eyeball and posterior nasal region, fig. 14 slightly posterior to the eyeball; fig. 12 through the anterior edge of the eyeball, fig. 11 through the middle of the same, and fig. 13 through the posterior edge. Fig. 11 corresponds approximately to fig. 10. × 12.5.

Fig. 15 Cross-section of head, right half, of Dermophis, about fifteen sections posterior to fig. 14. The section corresponds approximately to that of Herpele in fig. 16. × 7.5.

Fig. 16 Cross-section through the head, left half, of Herpele; section 171. Corresponds approximately to fig. 15. × 12.5.

tive olfactory epithelium. In brief, in the Amphibia in general, the posterior ventral olfactory glomeruli are related to Jacobson's organ, but not exclusively so. In Herpele and Geotrypetes, however, the posterior lateral glomeruli seem to be related exclusively to Jacobson's organ. Perhaps this is true of all the caecilians.

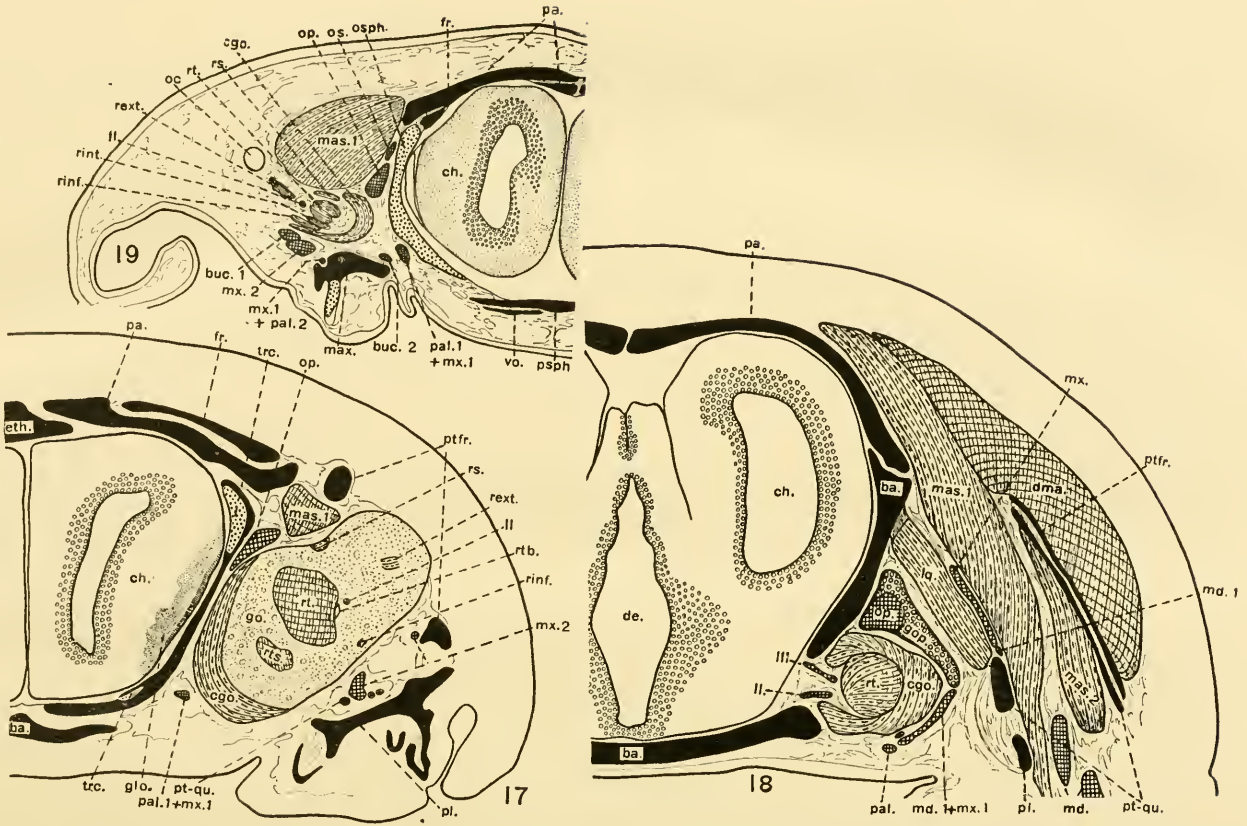
The path of the olfactory nerve in caecilians from the olfactory lobe to the point of distribution to the nasal organs is worthy of special notice. The olfactory nerves, dorsal and ventral, run, as described above, in tubular passages in the ethmoid bone. When a processus conchoides is formed, the ventral olfactory canal lies at the lateral border of a thin shelf-like extension of the ethmoid. The free edge of this shelf, containing the canal, is raised dorsally in such a way as to push in the ventral wall of the nasal chamber, dividing its cavity into medial and lateral portions (figs. 5 to 7, 11, 12; see also Wiedersheim, '79 pl. III and IV, figs. 32 to 34, 37 to 40; Sarasins, l.c., pl. XV, fig. 4). Anterior to the point where the nerve begins to leave the bony canal the vomer sends a dorsal extension to form a part of the support of the projecting ridge (figs. 6, 7, *vo.*) and more anteriorly still the nasopremaxillary sends a contributing element (fig. 5, *nas.-pmx.*). Jacobson's organ opens into the lateral part of the nasal chamber (figs. 6, 7, *jo.*). In Herpele, Caecilia, and Hypogeophis (Wiedersheim) the canal of the ventral olfactory nerve is developed in the base of the nasal septum and no processus conchoides of the ethmoid occurs. In consequence, the ventral wall of the nasal chamber is concave, not convex, and Jacobson's organ opens into the nasal chamber near its median border (figs. 8, 9, *jo.*). In Dermophis the ventral olfactory nerve supplies Jacobson's organ and the olfactory epithelium covering the convexity of the ridge on the floor of the olfactory chamber. The dorsal nerve supplies the medial, dorsal, and lateral walls. In Herpele the ventral nerve supplies Jacobson's organ and the epithelium of the floor; the dorsal nerve the medial and lateral walls. In both species the extreme lateral wall is not olfactory.

THE OPTIC AND EYE-MUSCLE NERVES

The rudimentary, or even vestigial, condition of the eyes in the caecilians is accompanied by a corresponding degeneracy of the muscles of the eyeball and related nerves, with certain exceptions. Two degrees of degeneration occur. In one, as in Dermophis, Geotrypetes, and Ichthyophis, the eye is covered by the skin; in the other, as in Herpele and Caecilia, the eye is situated beneath the maxilla. In Dermophis, Ichthyophis, and Geotrypetes the optic nerve, though rudimentary, may be traced from the eyeball through the mass of the tentacular, or orbital, gland, along the retractor muscle of the tentacle, thence through the membranous wall of the cranial cavity and to its entrance into the brain (figs. 14, 15, 17 to 19, *II*). In Herpele and Caecilia the eyeball is vestigial (fig. 10, *oc.*) and no trace of an optic nerve is found.

Of the eye-muscles and eye-muscle nerves in the caecilians various accounts have been given. Wiedersheim does not mention them. Waldschmidt describes an oculomotorius in Siphonops, which anastomoses with the ramus maxillaris V and supplies the compressor muscle of the orbital gland. Waldschmidt's oculomotorius is probably a branch of the ramus mandibularis V (*md. 1*) as will be shown later. He finds no trace of trochlearis or abducens. Burckhardt agrees with Waldschmidt as to the arrangement and innervation of the oculomotorius in Ichthyophis. He finds no other eye-muscle nerves. The Sarasins found in the embryo and adult of Ichthyophis four rectus and two oblique muscles, besides a large retractor tentaculi which they regarded as a modified retractor bulbi muscle. Of the innervation of these muscles they are uncertain. Leydig described four muscles of the eyeball in Siphonops. Marcus finds in the embryo of Hypogeophis all three eye-muscle nerves present, with their characteristic distribution. The fourth nerve is extremely attenuated; the sixth supplies the retractor tentaculi muscle, and the oculomotorius a levator bulbi muscle.

The writer finds in Dermophis four well-defined muscles attached to the eyeball, in two sets. An anterior pair has origin



Figs. 17 and 18 Cross-sections through the head, left half, dorsal part, of Geotrypetes petersii; fig. 17 immediately posterior to the eyeball, fig. 18 through the optic foramen. Fig. 17 corresponds approximately to fig. 14. × 37.5.

Fig. 19 Cross-section through the head, right half, dorsal part, of a late larval stage of Ichthyophis glutinosus; through the posterior edge of the eyeball. Corresponds approximately to figs. 14 and 17. × 37.5.

on the walls of the tentacular canal anterior to the eyeball (figs. 11, 12, *so.* and *io.*). The position and relations of these muscles indicate that they are the dorsal and ventral oblique muscles. The muscles of the second set originate close together posterior to the eyeball, from the fibrous envelope of the retractor tentaculi muscle and of the compressor muscle of the orbital gland (fig. 14, *rs.* and *rinf.*). They seem to correspond to the dorsal and ventral rectus muscles. Besides these four muscles a small muscle slip, originating near the point of origin of the preceding, runs to the posterior border of the eyeball, representing apparently a rectus lateralis (fig. 14, *rext.*). Along with the optic nerve, from the retractor tentaculi muscle to the eyeball, there runs a bundle of coarse fibers which seems like a rudimentary muscle, a retractor bulbi. Of a rectus medialis there seems to be no trace.

In Dermophis the eye-muscle nerves occur in their typical arrangement. As in all amphibians, when the eye is rudimentary, the fourth nerve is the least developed. The sixth, supplying the retractor tentaculi muscle, is the only one of considerable size. The oculomotorius was traced to within two or three sections of the origin of the muscle considered as the rectus dorsalis. The relation of the nerve to the rectus ventralis and the obliquus ventralis could not be determined, but is doubtless as has been described in other amphibians. Its innervation of a levator bulbi (compressor of the orbital gland), as stated by Marcus in Hypogeophis, was not found. An anastomosis of the oculomotorius with the ramus maxillaris V, such as was reported by Waldschmidt in Siphonops, certainly does not occur in Dermophis. The fourth nerve in Dermophis is extremely attenuated. It was traced some distance from its point of emergence from the brain along the medial border of the parietal bone, but was finally indistinguishable from the fibrous envelopes of the brain. The abducens nerve innervates the retractor tentaculi muscle and is correspondingly of considerable size. Owing to the poor differentiation of small nerves in the specimen of Dermophis, it was impossible to determine any relation of the abducens to the vestigial rectus lateralis muscle or to a retractor bulbi. In

Dermophis there exists a well-developed compressor muscle of the orbital gland (fig. 14, *cgo.*), consisting of an inner circular and an outer layer of longitudinal or oblique fibers. From the outer layer of the compressor muscle there runs posteriorly and laterally a muscle (figs. 14, 15, *tmpro.*) which has its other attachment on the tendon of the temporal muscle (*tm.*) In most of the species examined it is well nigh impossible to be sure that this muscle slip is not attached to the pterygoid process of the quadrate, along whose border it passes. In Caecilia, however, it is certain there is no such relation. While in Dermophis and Ichthyophis the anterior attachment of the muscle merges indistinguishably with the fibers of the compressor muscle of the orbital gland, in the other species examined, particularly Caecilia, the anterior connections are in close relation to the lateral wall of the skull in such a fashion that it is difficult to decide whether the attachment is to the latter, or to the mesial border of the sheath of the compressor muscle. Luther ('14, pp. 8, 69, figs. 1, 64) rightly interprets this muscle slip as an anterior head, 'caput preorbitale,' of the temporal (pseudotemporalis) muscle. In Geotrypetes, at least in the single specimen examined, there is complete lack of the temporal muscle, including this preorbital slip. The latter, when present, is always innervated by the same small branch of the ramus mandibularis V (*md.1*) which supplies the compressor muscle. The compressor muscle of the orbital gland has the characteristics of a levator bulbi muscle, as Luther (l.c.) has stated. As far as topographical relations go, it might be innervated by the oculomotorius, as Marcus states in Hypogeophis, but the strong development of the muscle in Dermophis does not accord well with the vestigial condition of the third nerve.

In Geotrypetes (fig. 17) the retractor tentaculi muscle is double, or, rather, there are two muscles, one the retractor tentaculi proper, with its origin in a fossa in the pterygoid process of the basal bone ventral to the Gasserian ganglion and insertion into the base of the tentacle, the other with origin from the sheath of the retractor tentaculi at the level of the optic foramen and insertion by a long slender tendon far anteriorly on the inner

border of the external sheath of the tentacle. The first is innervated by the abducens nerve, the second by the oculomotorius. The second muscle will be designated as the retractor muscle of the tentacular sheath (*rts.*). Six muscles, all nearly vestigial, are attached to the eyeball, in two groups. Originating from the sheath of the retractor tentaculi at its lateral ventral border is a muscle that has its insertion on the lateral posterior border of the eyeball, and evidently represents a rectus lateralis (*rext.*). A second one originating near the preceding, but from the sheath of the retractor of the tentacular sheath, is inserted on the ventral border of the eyeball and constitutes a rectus ventralis (*rinf.*). Inserted on the dorsal wall of the eyeball is a muscle that originates by a slender tendon from the mediäl border of the sheath of the retractor of the tentacular sheath, a rectus dorsalis (*rs.*). As in Dermophis, so in Geotrypetes, a delicate muscle slip follows the optic nerve from the retractor tentaculi to the eyeball, probably a retractor bulbi (*rtb.*). Two oblique muscles, as in Dermophis, arise from the dorsal and ventral walls of the tentacular canal and are inserted on the eyeball just anterior to the insertions of the dorsal and ventral rectus muscles. The faulty differentiation of nerve fibers in the specimen studied does not permit tracing the innervation of the vestigial ocular muscles. The position and innervation of the retractor of the tentacular sheath indicate that it represents a rectus medialis. This relation of the oculomotorius to the retractor muscle of the tentacular sheath clears up statements made by Waldschmidt that in Siphonops the compressor muscle of the orbital gland is innervated by the oculomotorius. In Geotrypetes, as the third nerve passes out through the optic foramen, it comes into close relations with the compressor muscle, and in an ordinary dissection might seem to end in the latter muscle (fig. 18). Marcus' statement that the third nerve innervated the levator bulbi (compressor) is not so easily explained, but is plainly an error. Waldschmidt figures an anastomosis between the ramus maxillaris V and the oculomotorius in Siphonops. In Geotrypetes the small branch of the ramus mandibularis V (*md. 1*) which innervates the compressor muscle of the orbital glands, runs, as in

Herpele and Dermophis, anteriorly along the ventral border of the ramus maxillaris V and passes medially to its innervation along an anastomosis between the ramus maxillaris V and the ramus palatinus VII, in such a manner as to give an appearance of the muscle being innervated by the ramus maxillaris V, or, as Waldschmidt interpreted it, forming an anastomosis between the ramus maxillaris V and the third nerve (fig. 18, *md.1* + *mx.1*). A trochlear nerve occurs in Geotrypetes, but its vestigial condition did not permit tracing it to its end in the dorsal oblique muscle, in the specimen examined.

In the larva of Ichthyophis at the stage examined by the writer the tentacle is not yet differentiated, and the retractor muscle of the tentacle has its insertion on the eyeball. The other ocular muscles are as described by the Sarasins (fig. 19). Of the eye-muscle nerves in Ichthyophis Burckhardt found only the oculomotor. The writers find the oculomotor and abducens, the latter innervating the retractor tentaculi. The trochlearis doubtless exists but it was not recognized.

The oculomotorius in the adult Ichthyophis is so vestigial that it probably was not seen either by Waldschmidt or by Burckhardt. The abducens passes through the skull much farther anteriorly than in other caecilians examined; and on emerging enters almost immediately into the base of the retractor tentaculi muscle, thus avoiding the common contact with the Gasserian ganglion. This extremely anterior exit of the abducens may have led to its being mistaken for the oculomotorius, or possibly overlooked entirely. The oculomotorius makes no anastomoses with other cranial nerves. The statements of Waldschmidt and Burckhardt to the contrary, i.e. that the oculomotorius anastomoses with the ramus maxillaris V, may possibly be explained by their having mistaken the anterior part of the ramus palatinus VII for an oculomotorius. But it seems more probable that their oculomotorius was an anastomosis between the ramus maxillaris and ramus palatinus, possibly containing the small motor twig of the ramus mandibularis V (*md.1*) which innervates the compressor muscle of the orbital gland. No vestige of a trochlearis was found in the adult Ichthyophis.

In Herpele and Caecilia the eye-muscles and eye-muscle nerves have completely disappeared, with the exception of the rectus lateralis muscle, represented by the retractor tentaculi muscle, and the abducens nerve innervating the latter muscle (figs. 8–10, 16, *rt.* and *VI*). The sixth nerve, in its anteriorly directed course after leaving the brain, comes into very close association with the ganglion of the ramus ophthalmicus profundus V. In Dermophis it is difficult to distinguish the nerve from the fibers of the ramus ophthalmicus profundus in the ganglion, but in Herpele it is clear that there is no anastomosis between the two nerves. This intimate association of the sixth nerve with the ganglion of the fifth nerve is not uncommon in the amphibians. In no instance has there been shown an anastomosis between the two nerves.

A compressor muscle of the orbital gland occurs in Herpele and Caecilia, but is not so extensively developed as in Dermophis, though the innervation is the same. The preorbital slip of the temporal muscle also occurs in these forms, but its anterior attachments are not so plainly related to the sheath of the compressor muscle. In Herpele it appears to be attached to the sheath of the muscle; in Caecilia, to the ventral trabecula. The condition in Caecilia seems to be the original one: origin on the trabecular region of the skull, insertion into the tendon of the temporal muscle.

Small muscles anterior to the adductor mandibulae group and innervated by the ramus mandibularis V have been reported and described in the Urodela. In Amphiuma and Siren (Norris, '08, '13; Bruner, '14 a and b; Luther, '14) a muscle, or muscles, innervated by the pterygoid branch of the ramus mandibularis V, and having its origin on the orbitosphenoid bone in Siren and on the pterygoid cartilage and maxilla in Amphiuma, is inserted on the antorbital cartilage. Norris recognized two muscles in Siren and Amphiuma and designated them as retractor and levator of the antorbital cartilage. Bruner more correctly surmised their evident respiratory function in their relation to the regulation of the size of the choana. Luther recognized but one muscle functionally and called it dilatator choanae. Luther believed

the dilatator choanae of Siren and Amphiuma and the compressor of the orbital gland in caecilians to be strictly homologous structures, and to be derived from a more primitive fundamental levator bulbi muscle, such as is found in many urodelans and anurans. Such an homology seems to the writers highly plausible, in spite of the fact that the innervation of the compressor muscle in the caecilians is by a nerve that also innervates the temporal and masseter muscles, derivatives of the primitive adductor mandibulae muscle, while the levator bulbi muscle is by theory derived from a primitive dorsal constrictor.

It is not quite clear to us what the function of the preorbital slip of the temporal muscle is when it is attached anteriorly to the compressor of the orbital gland. It may possibly assist the compressor muscle, but it more probably acts as a dilatator of the gland. In Dermophis the muscle is innervated by a branch that supplies the pterygoid as well as the masseter, temporal, and compressor muscles: In Herpele and Caecilia, by a branch arising from the base of the ramus mandibularis V near where the branch to the pterygoid muscle is given off. Its posterior connection with the tendon of the temporal muscle indicates that it is but a part of that muscle.

From this survey of the structures connected with the eyeball it becomes clear that there is nothing anomalous in the structure and relations of the ocular muscles and nerves in the caecilians other than that which results from extreme degeneration of most of the parts involved and the marked transformation which produces a retractor tentaculi muscle. All parts and their innervation can be directly homologized with structures and corresponding innervation in the urodele amphibians.

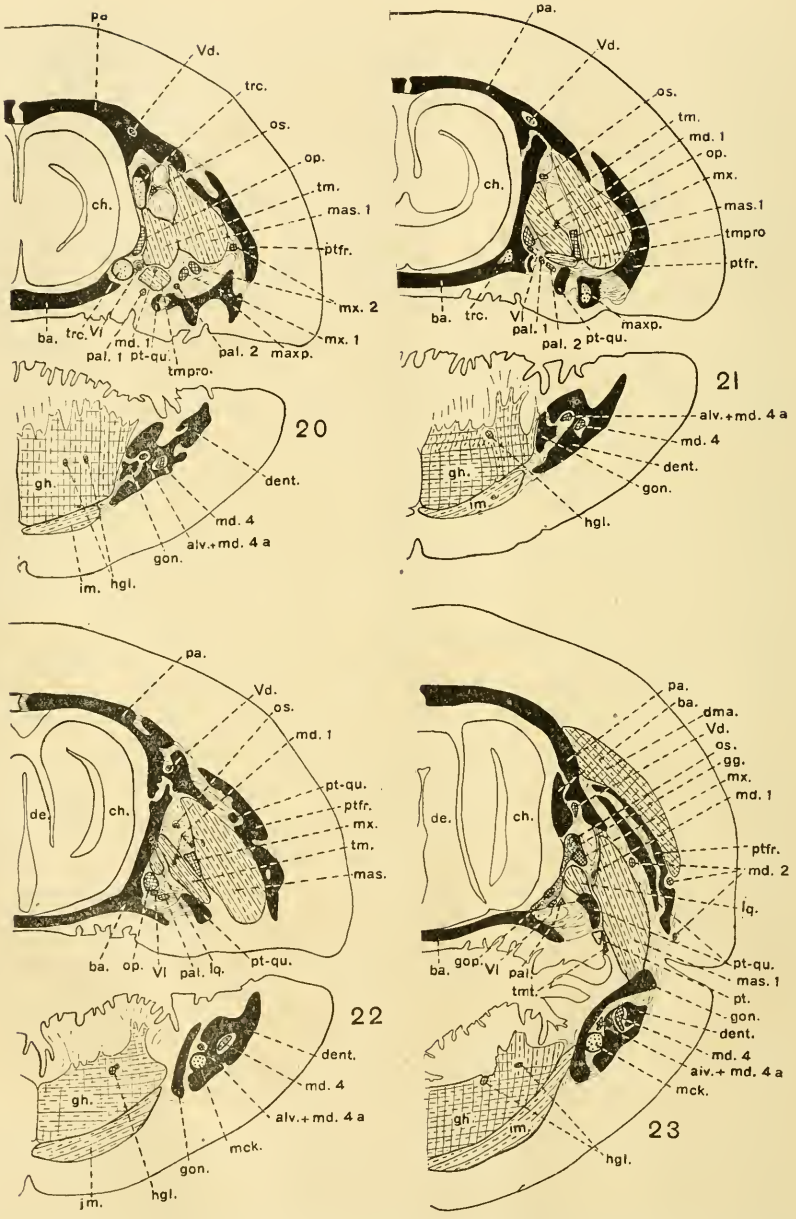
THE TRIGEMINAL NERVE

1. The roots and ganglia of the trigeminal nerve

The distinctly double nature of the fifth nerve in the caecilians was noticed by Wiedersheim and subsequent investigators. Brauer and Marcus agree that the ophthalmicus profundus ganglion arises from a dorsolateral placode, and the maxillo-mandibular ganglion from the neural crest.

In the adult condition of Herpele the two divisions arise from the brain by a common root which soon divides. A shorter lateral maxillo-mandibular division runs immediately into the Gasserian ganglion (figs. 25, 26, *Vrmd-mx.*). The latter is somewhat triangular in cross-section, and lies in a part of a long recess in the cranial wall which is occupied by the combined ganglia of the fifth and seventh nerves. The cavity is bounded posteriorly by the pterygoquadrate bone ventrally and laterally and by the alisphenoid region of the basal bone dorsally (figs. 25 to 29). Anteriorly the levator quadrati, temporal, and masseter muscles and the parietal bone have a share in its boundary (figs. 22 to 24). Posteriorly the Gasserian ganglion is in contact with the facial ganglion (figs. 27, 29, *gg.*, *gen.*), but distinct from it. Anteriorly it is slightly in contact with the profundus ganglion (figs. 23, 24, 29). Directly opposite the point of entrance of the root into the ganglion the trunk of the ramus mandibularis, composed of motor and somatic sensory fibers (figs. 25, 26, 44, *md.*) passes out laterally. A section through the ganglion at this level shows, at the medial ventral border of the ganglion, the ramus palatinus of the seventh nerve (*pal.*), with which fibers from the Gasserian ganglion are associated. A little farther ventromedially is the abducens nerve (*VI*). In the ganglion and a little dorsal to its middle is a small darkly colored strand of fibers (*os.*) derived, as will be described later, from the facial nerve. With it are apparently associated fibers from the fifth nerve. At the posterior dorsal border of the Gasserian ganglion this small strand has a small ganglion (figs. 27, 44, *glld.*), and its position seems to correspond to that of a dorsal lateral line ganglion in the larval stage. Toward the posterior part of the Gasserian ganglion there leaves its lateral border a small band of non-medullated fibers (fig. 27, *sy.V*), a part of the sympathetic chain, the course of which will be traced later.

The ophthalmic division of the root of the trigeminal nerve passes anteriorly at the lateral border of the brain (figs. 25, 26, *Vrop.*) and enters its ganglion at the anteroventral border of the Gasserian ganglion (fig. 24, *gop.*). For a short distance the two ganglia are in contact, but not confluent. At the lateral



Figs. 20 to 25 Cross-sections of the head, left half, of Herpele; through the main trunks and ganglia of the trigeminal nerve. Sections 185, 197, 209, 222, 226 and 234. × 12.5.

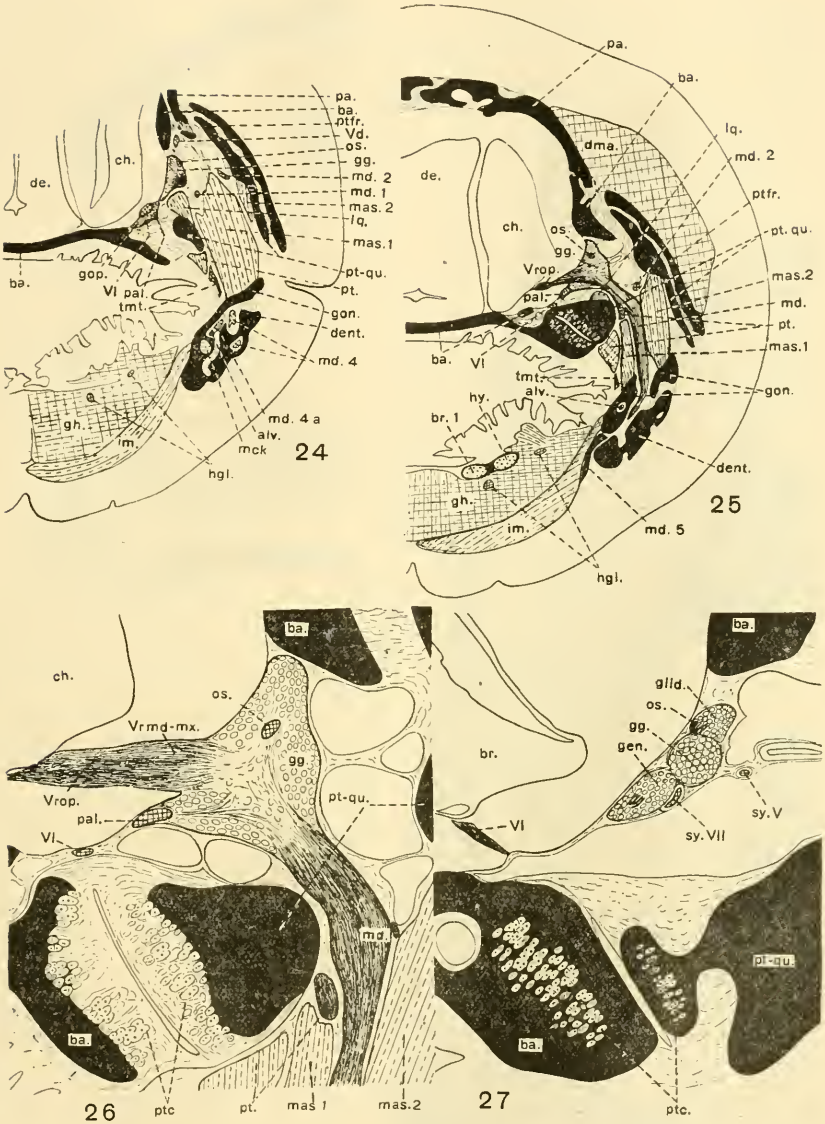


Fig. 26 Cross-section through the trigeminal nerve roots, the Gasserian ganglion and the mandibular trunk of Herpele; section 236. × 50.

Fig. 27 Cross-section through the geniculate, Gasserian and lateral-line ganglia, and the root of the abducens nerve of Herpele; section 244. × 50.

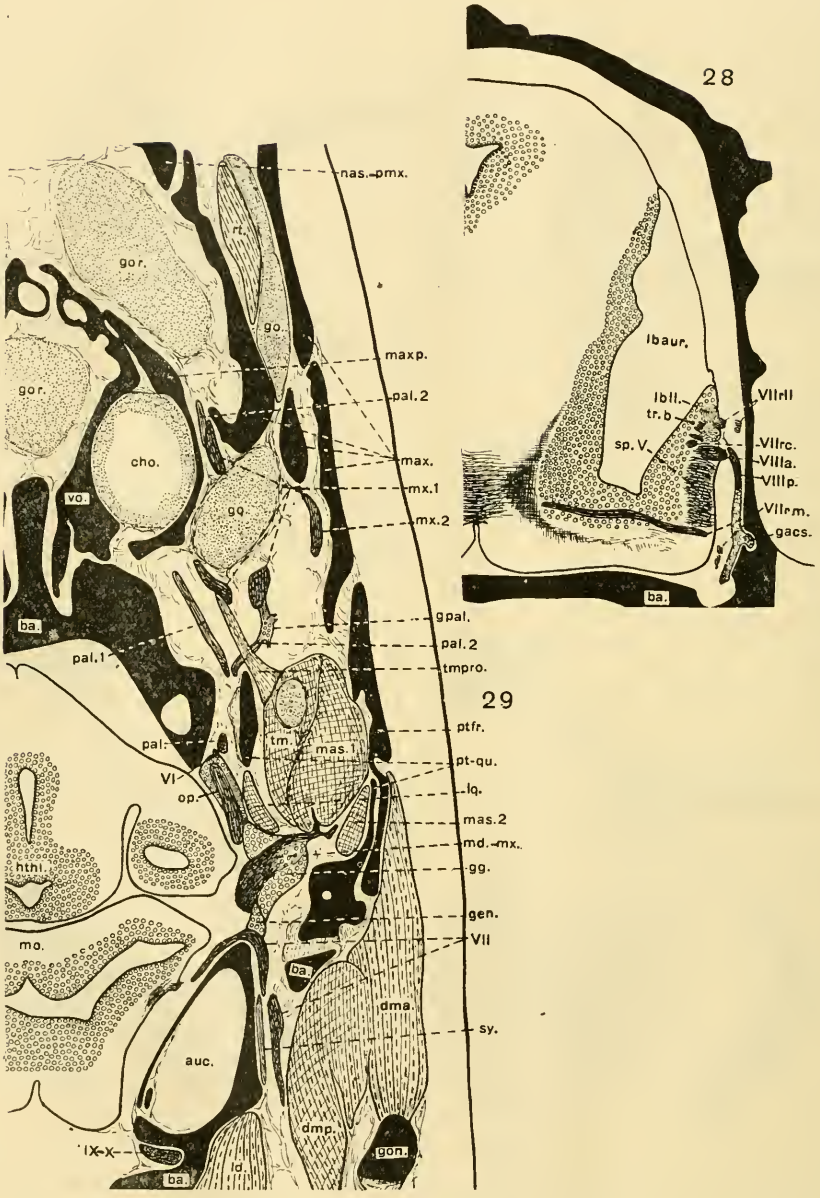


Fig. 28 Cross-section through the roots of the facial nerve of Herpele; corresponds to section 248. × 37.5.

Fig. 29 Horizontal section of the head, right half, through the trigeminal and facial nerve roots of Herpele. × 21.

ventral border of the ophthalmic are the abducens nerve and the ramus palatinus VII. The ophthalmic ganglion is somewhat lenticular concavoconvex in cross-section, the concavity toward the brain (figs. 23, 24, *gop.*) The ramus ophthalmicus profundus emerges from its extreme anterior end (figs. 22, 44, *op.*).

2. The ramus mandibularis V

As stated above, the nerve leaves the lateral ventral border of the Gasserian ganglion directly opposite the entrance of the root (figs. 25, 26, *md.*), passing out through a notch in the anterior border of the proximal part of the pterygoquadrate bone. Curving ventrally around the base of the pterygoquadrate, it passes between the anterior mesial and the posterior lateral divisions of the masseter muscle (figs. 25, 26) and then enters the lower jaw through a foramen in the dorsomedial border of the gonial bone. Within the jaw it divides into: *a*) a posterior ramulus intermandibularis which passes out through a foramen in the ventromedial part of the gonial to supply the intermandibular muscle and the skin overlying it (figs. 25. 44, *md.5*); *b*) a ramulus alveolaris (*md.4a.*) that soon unites with the ramus alveolaris VII (*alv.*), which has entered a canal in the gonial considerably farther posteriorly (figs. 24–20, 16, 15); *c*) the main nerve, ramulus mandibularis externus (*md.4*), that passes anteriorly in a space between the gonial and the dentary bones, soon in a canal in the dentary, in which it runs as far as the symphysis. The combination of the two alveolar nerves (*alv.* + *md.4a*), composed of somatic and visceral sensory fibers, runs at first along the dorsal border of Meckel's cartilage (figs. 23, 22); farther anteriorly, as the gonial decreases in size, in a groove in the medial border of the dentary (figs. 21, 20, 16, 15) as far as the symphysis. It and its branches are distributed to the bases of the teeth of the lower jaw, and presumably to the lateral epithelium of the floor of the mouth. The ramulus mandibularis externus innervates the skin overlying the lower jaw. In the larval stage of Ichthyophis the ramulus mandibularis externus V, runs not within the mandible, but along its lateral border, accompanied by the ramus mandibularis externus VII (r. mentalis externus).

Between the exit from the Gasserian ganglion and the entrance into the bony jaw, the ramus mandibularis gives off a number of small branches. As the nerve leaves the ganglion (from its very base or from the ganglion itself) a small nerve passes ventrally immediately into the levator quadrati muscle.

From the lateral border of the nerve trunk are two small branches given off to the anterior and posterior masseter muscles. A branch, given off from the anterior medial border, runs posteriorly into the pterygoid muscle (figs. 25, 26, 44, *pt.*). A short distance dorsal to the lower bony jaw there is given off, from the anterior border of the nerve, a somatic sensory branch to the skin in the region of the angle of the mouth. From the Gasserian ganglion at the very base of the ramus mandibularis a motor branch passes out which runs anteriorly along the dorsal border of the masseter muscle (fig. 24, 23, *md.1*). When the level is reached where the ramus maxillaris leaves the anterior end of the Gasserian ganglion this small nerve applies itself to the ventral border of the ramus maxillaris and divides into two parts (fig. 44), one running along the medial and the other along the lateral border of the ramus. Both nerves soon leave the maxillary trunk, the lateral one innervating the anterior masseter, the medial one supplying the temporalis and farther anteriorly the preorbital head of the latter muscle (figs. 21, 20, 16–14, *md.1*, *tmpro.*). Leaving the latter muscle, the medial nerve, at a level slightly anterior to the origin of the retractor muscle of the tentacle, curves dorsally around the medial border of the tentacular muscle to enter and innervate the compressor muscle of the orbital gland (fig. 16, *md.1*, *cgo.*). This nerve, *md.1*, is probably the one mistaken by Waldschmidt for an anastomosis between the oculomotorius and the ramus maxillaris V. It was doubtless this same small nerve which Wiedersheim described as derived from the ramus maxillaris and innervating mm. masseter and compressor of the orbital gland.

3. The ramus maxillaris V

The ramus maxillaris leaves the anterior end of the Gasserian ganglion at its lateral ventral border (fig. 23, *mx.*), and passes anteriorly and ventrally between the temporal and masseter muscles (figs. 22, 21). Having passed through the muscles it divides into medial and lateral branches (fig. 20, *mx.1*, *mx.2*). Shortly after this division the medial branch unites with a lateral division of the ramus palatinus VII (fig. 16, *mx.1* + *pal.2*) and at about the same place gives off a small branch, which running anteriorly and medially, unites with the medial division of the ramus palatinus (*pal.1* + *mx.1*). The union of the medial maxillaris with the lateral division of the ramus palatinus seems to be but temporary, with little or no mingling of fibers. Before leaving the medial maxillary branch the palatine element gives off a small nerve (fig. 16) which can be traced to the bases of the teeth situated on the maxilla. After leaving the maxillary branch the palatine element runs anteriorly, sending twigs to the choanal epithelium and the bases of the maxillary teeth, and presumably to the lateral oral epithelium (figs. 10, 9, *pal.2*). The nerve formed by the union of the medial palatine branch and the twig from the medial maxillary division (*pal.1* + *mx.1*) seems to innervate the medial wall of the choana, the roof of the mouth and the bases of the vomerine teeth (figs. 13–6, 4, 3, 44, *pal.1* + *mx.1*).

The main portion of the medial branch of the ramus maxillaris (*mx.1*) continues anteriorly along the dorsal border of the palatine process of the maxilla and takes a position in a groove in the latter along with the lateral palatine branches to which reference was made above. This groove, farther on opens ventrally, and then becomes a closed canal in the palatine process (figs. 10, 12). The canal next becomes a groove opening into the medial ventral part of the tentacular canal (figs. 8, 9, 11), and farther anteriorly becomes a canal again (figs. 4–2), in which the nerve continues without important branches to the region of the tentacular pore, where it is distributed to the sheath of the tentacle and to the skin of the ventral and ventrolateral surfaces of the snout

in the vicinity of the tentacle (fig. 2). At its final terminations there is much commingling and some anastomosing with fibers of the ramus ophthalmicus profundus V. The lateral branch of the ramus maxillaris (figs. 20, 16, 14–8, *mx.2*), which has no anastomoses with the ramus palatinus VII, is distributed to the skin at the side of the head.

In Ichthyophis (fig. 19) the lateral division of the maxillaris (*mx.2*) is the main nerve. The whole of the small medial division (*mx.1*) unites with a medial palatine branch (*pal.1*), the combined nerve having a distribution similar to the corresponding nerve in Herpele. As in Herpele, a lateral palatine branch (*pal.2*) unites temporarily, if at all, with the maxillaris.

In Dermophis the main maxillary nerve divides into a smaller lateral and a larger medial portion. The lateral division passes out to the skin. Two anastomoses of the medial branch occur, much as in Herpele.

The ramus maxillaris in Geotrypetes does not divide into main medial and lateral branches, but from it there passes ventrally, medially, and posteriorly around the lateral border of the orbit, a branch that unites with the ramus palatinus VII. Along this anastomosis there also passes, as mentioned in a previous section (p. 508) a small branch of the ramus mandibularis V (*md.1*) to innervate the compressor muscle of the orbital glands (fig. 18, *md.1* + *mx.1*). At the point where the maxillary branch joins the ramus palatinus, from the latter a small branch (*pal. 2*) runs laterally and anteriorly to join the main maxillaris. In this way there is formed a double anastomosis of palatine and trigeminal elements so characteristic of the Urodela. In the latter, however, the trigeminal component is from the ophthalmicus profundus and not from the maxillaris. The posterior maxillo-palatine anastomosis in Geotrypetes grazes the lateral border of the profundus ganglion, and possibly a similar condition in Ichthyophis and Siphonops was mistaken by Wiedersheim for an anastomosis between the maxillaris and profundus. The presence of a motor element (*md.1*) in the anastomosis may explain the supposition of Wiedersheim that the compressor muscle of the orbital glands was innervated through the ramus maxillaris.

4. Small nerves arising from the Gasserian ganglion

Besides the two main trunks from the Gasserian ganglion there are a few small nerves that require mention. One of these arises from the lateral border of the ganglion nearly dorsal to the exit of the ramus mandibularis. It is somatic sensory and, after dividing within the outer cranial wall, its two branches run anteriorly, pass around the anterior border of the external expansion of the pterygoquadrate bone, and go out through foramina in the postfrontal bone, the larger ventral branch sending twigs anteriorly and posteriorly to the skin in the region of the angle of the mouth, the smaller dorsal division going anteriorly (figs. 25–23, *md.2*). In other amphibians (Amphiuma, Siren) a somatic sensory branch of the ramus mandibularis has a similar distribution.

At about the same level with the preceding nerve, but arising from the dorsal side of the ganglion, a second sensory nerve (figs. 23–20, 44, *Vd*) passes immediately dorsally between the ventral border of the parietal bone and the dorsal trabecular bone, then through a canal in the parietal. It divides in the canal and, on emerging from the skull, is distributed to the skin in the dorsolateral region. This is a nerve which in amphibians generally is referred to by writers as 'dorsal fifth.'

Directly dorsal to the exit of the ramus maxillaris a small nerve passes anteriorly and slightly dorsally which merits special consideration. As will be noticed later, the facial nerve contributes a dorsal component (*VII lld.*) to the fifth nerve, which can be traced from the brain through the root of the seventh nerve (fig. 28) into what appears to be a distinct ganglionic mass on the posterior dorsal border of the Gasserian ganglion (figs. 27, 44, *glld.*). From this small mass of ganglion cells a nerve strand (*os*) passes anteriorly into the Gasserian ganglion proper. This is sharply differentiated by its much darker color from the fiber tracts of the trigeminus (figs. 26–23). After leaving the Gasserian ganglion it runs anteriorly, dorsal to the ramus maxillaris, in the space between the temporal and masseter muscles, taking a position at the lateral edge of the orbitosphenoid region

of the basal bone and farther anteriorly at the lateral border of the dorsal trabecular cartilage bar (figs. 22–20, 16). It continues unbranched as far as the level of the choana. There a branch of the ramus ophthalmicus profundus joins it in such a way as to render it difficult to follow the facialis fibers with certainty. Along with twigs of the fifth nerve, they are soon distributed to the skin of the dorsal lateral part of the head in a region slightly anterior to the position of the eye (figs. 10–8, *op.1* + *os*). The significance of this nerve will be discussed later in connection with the facialis.

5. The ramus ophthalmicus profundus V

As the ramus ophthalmicus profundus passes out of the ophthalmic ganglion, the abducens nerve lies at its ventral border and continues with it anteriorly, but gradually separating from it, until the retractor muscle of the tentacle is reached (figs. 22–20, *op.,VI*). The ramus ophthalmicus profundus at first runs close to the lateral border of the orbitosphenoid region of the basal bone (figs. 22, 21), then, farther anteriorly, in the same relation to the membrane which closes the great intertrabecular foramen (figs. 20, 16), and to the bony trabecular wall. As it passes anteriorly it gradually rises dorsally, giving off no branches until the level of the choana is reached. Here a large nerve (*op.1*) is given off, a branch of which comes into close relationship with the nerve derived from the facialis (*os.*), as described in the preceding section (figs. 10–8). Soon after giving off the larger nerve the ramus ophthalmicus profundus enters a canal in the dorsal part of the ethmoid bone (fig. 10) and continues in it until the level of the anterior end of the olfactory lobe is reached. There the nerve continues in the dorsal part of the nasal chamber on the ventral border of a lateral wing-like extension of the ethmoid (fig. 8, *op.2*), and farther anteriorly along the medial ventral border of the dorsal portion of the nasopremaxilla (figs. 4–2), and finally in a canal in the same bone to its distribution to the skin of the tip of the snout (fig. 1).

On its course from the ganglion to its final distribution the ramus gives off comparatively few branches. The first branch,

at the level of the choana as stated above, is a large nerve (*op.1*) one or more of whose twigs interweave indistinguishably with the small nerve sent forward through the Gasserian ganglion from the facialis. The main portion of this first branch (*op.1*) continues anteriorly and laterally, soon running in a groove and canal in the frontal bone (fig. 10), then between the frontal and the maxilla (fig. 9), and eventually in the maxilla at the lateral dorsal border of the nasal chamber (fig. 8), and thence on the dorsal wall of the tentacular canal (figs. 4, 3), from which course it sends out cutaneous branches to the side of the head, being finally distributed to the tentacular sheath and to the skin in the region of the tentacle, its fibers commingling and anastomosing with the fibers of the medial branch of the ramus maxillaris. The sensory innervation of the tentacle itself is from this lateral branch of the ramus ophthalmicus profundus, and not from the facialis, as stated by Marcus.

A second large branch (*op. 3*) leaves the main trunk as the latter is passing through the canal in the dorsal ethmoidal region, and passes anteriorly along the dorsal wall of the nasal chamber (figs. 9, 8), farther anteriorly along the dorsolateral wall (figs. 4, 3), just in the ventral border of the frontal bone (figs. 2, 1). It possibly supplies Jacobson's gland along whose border it runs, but its chief distribution is to the skin on the side of the head dorsal to the tentacular pit, and thence as far anteriorly as the nostril. At about the level where the ventral olfactory nerve leaves its canal in the ethmoid bone to disperse to the olfactory epithelium, there leaves the ramus ophthalmicus profundus (now designated as *op.2*) a minute nerve, or two that soon unite, which runs far anteriorly along the dorsal olfactory epithelium (figs. 4–1, *op.2b*). It divides anteriorly and finally disappears at the border of the olfactory epithelium, to which it is evidently distributed. Near where the dorsal olfactory nerve breaks up into its chief branches a branch (*op.2v*) is given off from the ventral side of the ramus ophthalmicus profundus which, for a short distance, applies itself closely to one of the chief branches of the dorsal olfactory nerve, then turns abruptly ventrally at the medial border of the olfactory epithelium, and

passes into a canal at the medial border of the nasopremaxilla on the ventral wall of the nasal chamber (figs. 3–1). After some anastomoses with terminal branches of the medial ramulus of the ramus maxillaris V, it is distributed to the ventral epithelium of the snout, anterior to the tentacular pit. From the point of origin of the last-named nerve (*op.2v*) anteriorly there are given off from the main ramus numerous twigs to the skin on the top of the head and snout. The final destination of the main ramus (*op.2*) is to the skin of the ventral side of the tip of the snout.

6. General reflections upon the trigeminal nerve

The distinctness of the Gasserian and profundus ganglia from each other is apparently accompanied by a sharper delimitation of the peripheral portions of the nerves than occurs in other amphibian groups. The almost complete absence of lateral-line components in the adult condition makes for greater independence of the fifth and seventh nerves, and produces a simpler structure. Of significance is the lack of an anastomosis between the ramus ophthalmicus profundus V and the ramus palatinus VII, and the presence of an anastomosis between the maxillaris V and the palatinus VII. In the Urodela the former anastomosis occurs; in the Anura both types occur. In Siren, as the writer has pointed out ('13), somatic sensory fibers pass from the maxillaris V into the palatinus VII.

The ramus maxillaris in caecilians is significantly large as compared with the corresponding nerve in Urodela. Its anastomoses with the ramus palatinus suggest that it carries fibers which in the Urodela run in the ramus ophthalmicus profundus. The distinctness of the profundus from the Gasserian ganglion in caecilians may be responsible for this seeming transfer of the palatine anastomosis from the profundus to the maxillaris. The lateral division of the ramus maxillaris in caecilians has about the same distribution as the entire ramus maxillaris in the urodeles; the medial branch which contributes to the palatine anastomosis in the caecilians is distributed to territory that belongs

to the ramus ophthalmicus profundus in the urodeles. Even in the caecilians the terminal twigs of the medial maxillaris anastomose with twigs of the profundus.

In the Urodela the writer ('13) finds the ramus mandibularis V to have the following characteristic branches: 1) small short motor branches to the temporal and posterior masseter muscles as the main nerve passes through the muscles; 2) a large sensory branch (*md.2*) supplying the skin in the region of the angle of the jaw; 3) a motor branch (*md.1*) supplying the pterygoid and anterior masseter muscles and sending an anterior division into the antorbital muscles; 4) a sensory branch, or branches (*md.3*) innervating the skin overlying the jaw in the region of the origin of the main nerve; 5) the main mandibular nerve (*md.4*) supplying the skin overlying the greater part of the lower jaw, with an alveolar branch (*md.4a*) that unites with an alveolar branch of the facialis; 6) a posterior branch (*md.5*) which supplies the intermandibular muscles and the overlying skin. All these except *md.3* are distinctly differentiated in the caecilians.

In the Urodela the ramus ophthalmicus profundus shows four characteristic divisions: an ophthalmicus profundus minor (*op.1*), given off near the ganglion and supplying the top of the head, and three terminal divisions, medial, lateral, and ventral. The medial division, nasalis internus (*op.2*), supplies the snout region dorsally; the lateral division, nasalis externus (*op.3*), supplies the side region of the snout, and the ventral (*op.4*) forms the anastomosis with the ramus palatinus VII. The latter division is absent in caecilians, unless the branch designated as *op.2v* represents it. An ophthalmicus profundus minor (*op.1*) is doubtfully present in caecilians. Posteriorly it may be represented by the dorsal sensory branch (*Vd.*) arising from the Gasserian ganglion, and anteriorly by the anastomoses of the ramus ophthalmicus profundus with the facialis ramus (*os.*). The nerve in the caecilians designated by the writer as *op.1* only in part corresponds to an ophthalmicus profundus minor, for its lateral extension seems to represent in part a nasalis externus.

THE FACIAL NERVE

1. Roots and ganglia of the facial nerve

Three distinct elements are discernible in the root of the facialis: a motor root of deeply medullated fibers (*VIIrm.*) which emerges on the ventral lateral border of the auricular lobe of the medulla, a visceral sensory lightly medullated component (*VIIrc*) which enters the medulla closely associated with fibers of the auditory nerve, and a third distinctly but not heavily medullated element (*VIIrll.*) that enters the medulla farther dorsally than the other components (figs. 28, 44).

In the lateral wall of the medulla in the Urodela, at the origin of the VII–VIII nerves, a region occurs, dorsal to the heavily medullated tractus spinalis trigemini, which may be termed the acusticum. In it are three longitudinal columns of white matter. The roots of the auditory nerve are connected with the ventral of these, and associated with it is a small medullated tract, 'tractus b' of Kingsbury ('95) ,which a posterior auditory rootlet enters. Through this ventral column of the acusticum the communis root of the facialis passes transversely to enter the fasciculus communis. With the middle column of the acusticum the second and third lateral line roots of the facialis are connected. The dorsal column, 'dorsal island' of Kingsbury, receives the first lateral-line root of the seventh nerve.

In Herpele there are two longitudinal fiber columns in the acusticum. Into the ventral of these the fibers of the auditory nerve enter, and with it is associated a small medullated tract, into which some posterior root fibers of the same nerve enter. The communis root of the facialis passes through this ventral column, closely associated as in the Urodela, with the anterior root of the auditory nerve (fig. 28). The dorsal of the two columns of the acusticum receives the fibers of the dorsal root of the facialis above mentioned. A true lateral line lobe is absent in the caecilians, but this dorsal alba seems to correspond to the column with which the second and third lateral-line roots in the Urodela are associated. That is, in its relation to the other components of the facialis, in its entrance into the medulla,

and in its ganglionic relationships, this third element of the facialis is beyond question lateral line in character. It passes along the posterior dorsal border of the geniculate ganglion without entering it, but does appear to exchange fibers with it. It enters a small ganglionic mass on the posterior dorsal border of the Gasserian ganglion (figs. 27, 44, *glld.*) and appears to become ganglionated at once, as a few sections anteriorly it becomes a compact strand, which is maintained during its course through the Gasserian ganglion.

In the larva of Ichthyophis the seventh nerve has a full lateral-line complement. A lateralis root enters the dorsal part of the acusticum. There are two distinct lateral-line ganglia on the facialis; the anterior of these, from which the rami ophthalmicus superficialis and buccalis arise, lies dorsal to the Gasserian ganglion; the other ganglion is situated at the posterior dorsal border of the Gasserian ganglion, in contact with the dorsolateral border of the geniculate ganglion (fig. 30).

The geniculate ganglion is a small oval-shaped structure, in contact with the Gasserian ganglion anteriorly, but easily distinguished from it. Four groups of fibers leave it: a ramus palatinus leaves its anterior end; a ramus hyomandibularis of motor and visceral sensory fibers passes from it posteriorly; a sympathetic element runs posteriorly, closely associated with the preceding, to unite with the sympathetic branch from the Gasserian ganglion to form the main sympathetic trunk; fibers, their character unknown, join the lateral-line strand dorsally. In Dermophis the geniculate ganglion does not come in contact dorsally with the Gasserian ganglion.

2. The ramus palatinus VII

This leaves the extreme anterior end of the geniculate ganglion and then appears at the medial ventral border of the Gasserian ganglion. In a section through the entrance of the trigeminal root into the Gasserian ganglion (fig. 26) a distinct tract of fibers is seen joining the ramus palatinus. From this point the ramus palatinus supposedly contains somatic sensory as well as visceral

sensory fibers. It continues at the ventral medial border of the Gasserian ganglion until the profundus ganglion is reached (fig. 24). From this point the ramus palatinus and the abducens nerve, which has become closely associated with it, run at the medial ventral border of the latter ganglion. At about the level where the ramus maxillaris V emerges ventrally from the masseter and temporal muscles the ramus palatinus divides into

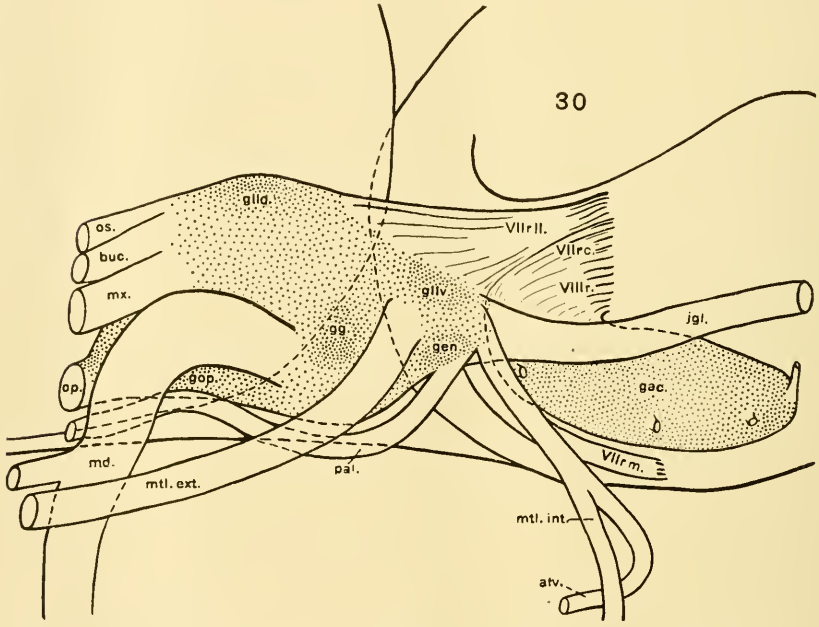


Fig. 30 A projection upon the sagittal plane of the V–VII–VIII ganglia with their chief nerve trunks, of a late larval stage of Ichthyophis glutinosus. × 55.

medial and lateral branches. The anastomoses of these branches with the ramus maxillaris have been noticed in the section describing the latter nerve.

In Ichthyophis the ramus palatinus passes at first directly ventrally from the ganglion in a canal in the basal bone, then, turning abruptly anteriorly, emerges from the canal and runs anteriorly along the usual course.

Just before the lateral branch of the ramus palatinus (*Pal.2*) unites with the medial division of the ramus maxillaris (*mx.1*), a small ganglion (figs. 29, 44, *gpal.*) occurs on the former. This ganglion is so much flattened horizontally that in a cross-section of the head it is detected with difficulty. From the ganglion small nerves proceed, one anterolaterally and the other posterolaterally. In Dermophis and the larva of Ichthyophis a palatine ganglion occurs in essentially the same relationships as in Herpele. Apparently it is absent in Geotrypetes.

In Amblystoma, according to Coghill, there is a ganglion on the ramus palatinus at the junction of its lateral division with the lateral division of the trigeminal element in the palatine-trigeminal anastomosis. Norris found in Amphiuma scattered ganglion cells just before the anastomosis is reached.

3. The truncus hyomandibularis VII

This trunk passes directly posteriorly from the geniculate ganglion in a space between the anterior ventral edge of the auditory capsule and the suspensorium (fig. 29, *VII*). When the stapes is reached the nerve takes a position on the dorsal border of the latter, at the ventrolateral border of the ear capsule. The two sympathetic nerves from the Gasserian and geniculate ganglia unite into a single trunk and the latter joins the ramus hyomandibularis, running first along its dorsal and then its medial border, soon becoming sharply distinct from the ramus hyomandibularis. The first branch given off from the hyomandibular ramus is a motor nerve to the anterior part of the depressor mandibulae muscle (fig. 44, *dma.*) almost immediately followed by a visceral sensory nerve (*alv.*) which curves ventrally around the lateral border of the stapes, along the medial border of the depressor mandibulae muscle, and, reaching the lower jaw, enters a canal in the gonial bone, in which, as the ramus alveolaris VII, it runs anteriorly to its junction with the ramulus alveolaris V, as previously described. A short distance posterior to the ramus alveolaris a second branch is given off to the depressor mandibulae muscle, innervating the

posterior part of the muscle (*dmp.*). The remainder of the hyomandibular nerve, the ramus jugularis (*jgl.*) then passes posteriorly at the lateral border of the ear capsule. Posterior to the latter the nerve runs slightly lateral to the vagus nerve, nearly paralleling its course, and remains undivided until the level of the first great sympathetic ganglion is reached (figs. 32 to 34, *VII*). At this place the jugularis begins to divide. First to be given off is a branch which runs anteroventrally into a muscle that may be considered as the equivalent of the interhyoideus muscle of the Urodela. Anteriorly this muscle is in contact with the intermandibular muscle, but sharply distinct from it. The fibers are attached at one end to the lateral border of the gonial bone and at the other along the medial ventral raphe (figs. 32, 33, *ih.*). Farther posteriorly the fibers assume a more oblique direction, attached anteriorly and dorsally to the longitudinal tendon which runs posteriorly from the gonial, and posteriorly and ventrally to the fasciae covering the underlying thoracicohyoideus muscles, and finally assuming a longitudinal direction parallel with the fibers of the omo-humero-maxillaris (sphincter colli) muscle, with which muscle there is posteriorly an indistinguishable blending (figs. 34, 38 to 40, *sphc.*). As stated above, the anterior part of the interhyoideus is innervated by a branch of the ramus jugularis. The posterior part of the muscle is supplied by other and posteriorly directed branches of the same nerve. Posteriorly directed branches also innervate the omohumero-maxillaris muscle. The latter is plainly the homologue of the levator maxillae inferioris ascendens (Fischer) of Amphiuma and the sphincter colli of other urodeles and of reptiles. Together with part of the interhyoideus, it constitutes an extraordinarily elongate visceral arch muscle, extending posteriorly into the trunk beyond the level of the sixth spinal nerve. Correspondingly, there is an unusual posterior extension of the branches of the ramus jugularis.

In the larva of Ichthyophis the ramus alveolaris VII arises from the ganglion along with a mentalis internus, and not with the ramus jugularis (fig. 30). In the adult it leaves the ganglion as a distinct nerve.

The jugularis in the larva of Ichthyophis also innervates a levator hyoidei and a ceratohyoideus externus muscle (fig. 41, *lh.*, *che.*).

In Dermophis the muscles innervated by the ramus jugularis have much the same relative proportions as in Herpele. In Geotrypetes there is a sharp distinction between the interhyoideus and omo-humero-maxillaris muscles.

4. The ramus ophthalmicus superficialis VII

As stated above (pp. 524, 525) there is in the root of the facialis nerve a tract of fibers which enters a dorsal region of the medulla and seems to represent a lateral-line center (fig. 28). This tract becomes ganglionated in a small mass of cells on the posterior dorsal border of the Gasserian ganglion, but seems to receive fibers from the geniculate ganglion. Its course through the Gasserian ganglion is easy to follow. As described in a previous section (p. 520), it anastomoses peripherally with branches of the ramus ophthalmicus profundus, and is evidently distributed to the skin. Although no neuromasts were found, the nerve, from its connections and relationships, is beyond question a representative of the ramus ophthalmicus superficialis. The fibers which it receives from the geniculate ganglion are supposedly visceral sensory, but their special distribution is unknown. In Dermophis the geniculate and Gasserian ganglia are not in contact, but this ramus bridges over the gap between them. In Geotrypetes it is wanting.

Waldschmidt describes and figures in Epicrium (Ichthyophis) a small cutaneous nerve which he believes to arise from the Gasserian ganglion, and which is undoubtedly this ramus ophthalmicus superficialis. Waldschmidt's commissure between the geniculate and Gasserian ganglia is probably this same nerve passing from the facialis root into the Gasserian ganglion. Marcus describes a ramus ophthalmicus superficialis in the trigeminus of Hypogeophis.

5. Lateralis components in the facial nerve of the larva of Ichthyophis

As shown in figure 30 (a projection of the V–VII–VIII ganglia of a larval Ichthyophis), there are two lateral-line ganglia in the complex, an anterior dorsal (*glld.*) and a posterior ventral (*gllv.*). From the first the rami ophthalmicus superficialis (*os.*) and buccalis (*buc.*) pass anteriorly; from the second there also run two nerves, a larger anterior mentalis externus (*mtl.ext.*), which supplies the oral series of neuromasts and accompanies the main ramulus mandibularis externus V along the lateral border of the lower jaw, and a posterior mentalis internus (*mtl.int.*) which supplies the gular series of neuromasts of the lower jaw. The ramus alveolaris VII passes out of the ganglion with the latter nerve. The ramus buccalis, at the level where the ramus maxillaris V divides into lateral and medial divisions, sends off a medial brauch (*buc.2*) which runs anteriorly, closely paralleling the course of the combined medial maxillary and palatine branches (*pal.1* + *mx.1*). It supplies neuromasts on the tip of the snout (figs. 19, 43). In Siren a strictly comparable branch occurs, a branch of the buccalis (*buc.2*) which passes ventrally (together with cutaneous fibers of the maxillaris) into the palatine-ophthalmic anastomosis, but quickly separates to run to neuromasts on the tip of the snout.

The structure and relationships of the lateralis ganglia and nerve trunks in a larval caecilian (fig. 43) are in essentials like those in the Urodela.

THE AUDITORY NERVE

The fibers of the auditory nerve enter the brain in close association with the visceral sensory root of the facialis. Although the otic capsule and the internal ear have the form and arrangement of the fully developed ear in the Urodela, it is evident that the auditory ganglion is more or less rudimentary. It consists of an anterior transversely situated mass of cells, constituting apparently a vestibular ganglion, continuous posteriorly with a longitudinal vertical, extremely thin ganglionic mass, a saccular

ganglion (fig. 28, *gacs.*). None of the ganglionic cell mass enters the ear capsule, in which respect there is a marked divergence from the condition in the urodeles. The anterior part of the ganglion extends from the brain wall postero-laterally out to the medial wall of the ear capsule. The lateral-line and visceral-sensory components of the facialis run along in and through the anterior border of this vestibular ganglionic material. The nerve which supplies the sense organs of the utriculus and the anterior and horizontal ampullae is given off from the lateral end of this anterior ganglion. From the medial, brain end of the anterior ganglionic mass the remainder of the acoustic ganglion extends posteriorly as a thin vertical plate of cells, somewhat crescentric in cross-section. From this part of the ganglion are given off five nerves, four ventral ones to the sacculus and one dorsal to the macula neglecta, to the ampulla of the posterior canal, and to a sense-organ in the posterior part of the utriculus which seems to be peculiar to caecilians.

THE GLOSSOPHARYNGEAL AND VAGUS NERVES

1. The roots and ganglia of the IX–X complex

As in other Amphibia, the ninth and tenth nerves are so closely associated that it is difficult to distinguish their proximal portions from each other. In Herpele the two nerves arise from the brain by five roots or groups of rootlets. Four of these, which represent the vagus, unite into a single one, ventral to which runs a smaller or glossopharyngeal root. In Geotrypetes the exact composition of these roots was determined with precision. The glossopharyngeal root arises, as in other Amphibia, by two rootlets, visceral sensory fibers that enter the fasciculus communis, and visceral motor. The first and second vagus roots contain both visceral sensory and visceral motor elements; the third and fourth roots are exclusively motor. Comparison of this condition with that in other amphibians shows that in the Urodela in general there are four groups of rootlets in the IX–X complex: 1) lateral-line root fibers (absent in adult caecilians); 2) visceral sensory and motor root fibers of the glossopharyngeus;

3) visceral sensory, somatic sensory, and visceral motor rootlets, often in two or more roots, vagus roots proper; 4) motor fibers, forming an accessory group. In the caecilians the first and second vagus roots correspond to the third urodele group, but the somatic sensory fibers are absent; the third and fourth roots form the accessory element of the vagus. In the frog tadpole Strong ('95) shows four roots of the vagus exclusive of the lateral line element. The anterior of these is visceral sensory and motor; the second is visceral sensory, somatic sensory, and visceral motor; the third is motor with a small visceral sensory contingent; the fourth is exclusively motor.

In the larva of Ichthyophis a lateral line root of the vagus enters the dorsal part of the medulla.

The absence of a somatic sensory component from the IX–X complex is noteworthy.

In Herpele the IX–X roots run out to the mesial wall of the ear capsule and pass back in a groove at the mesial-ventral border of the posterior semicircular canal (fig. 33, *IXr.*, *Xr.*), thence laterally around the posterior border of the latter and dorsal to the wall of the posterior extension of the sacculus. Close to the emergence from the skull is a small ganglion, usually on the posterior border of the nerve roots, which evidently belongs to the vagus nerve (figs. 32, 44, *g.Xr.*). The glossopharyngeal and vagal nerve roots in Herpele are so closely joined that it is difficult to distinguish one from the other as they pass out of the skull. When a thin layer of connective tissue separates them it is possible to trace the fibers of the ninth nerve out past the small ganglion mentioned above and into a ganglion situated dorsal to the sympathetic chain and anterolateral to a second and larger ganglion on the vagus trunk (fig. 44, *ggl.*, *g.Xtr.*). There is thus seen to be two ganglia upon the vagus, a small ganglion near the exit of the nerve, and a larger elongate one farther laterally dorsal to the sympathetic chain. The first, for want of a more exact term, may be called the root ganglion and the second the trunk ganglion. In some instances the roots of the ninth and tenth nerves are so closely united that the glossopharyngeal and vagus trunk ganglia are indistinguishably fused

together (fig. 34, *ggl.* + *g.Xtr.*). The occipital nerve (*ocn.*) runs across and through the posterior border of the root ganglion of the vagus in such a way as to give an appearance of a sixth root (figs. 32, 44.

In the single specimen of Dermophis examined there were nine rootlets in the IX–X complex. Six of these unite to form the larger dorsal vagal root, and from the other three comes the smaller ventral glossopharyngeal root (fig. 36). On one side of the specimen the ninth and tenth ganglia are not in contact. The smaller root enters an anterior ganglion (*ggl.*) and the larger root a posterior one (*gv.*). There is but one ganglion (an elongate one) on the root of the vagus in Dermophis, evidently representing a fusion of the root and trunk ganglia in Herpele. The occipital nerve passes around the posterior border of the vagal ganglion without contact with it. From the glossopharyngeal ganglion a nerve passes (figs. 31, 36, *anast.IX* + *sy.*) into the sympathetic ganglion.

Fischer figures and describes ('43, p. 42, pl. III, fig. 2) in Siphonops a nerve passing from the vagus into the great sympathetic ganglion. Wiedersheim ('79, pl. VII, fig. 80) shows in Ichthyophis a nerve passing from the vagus into the sympathetic trunk. Waldschmidt ('87, pl. XXXI, fig. 32) evidently saw a similar structure. In Herpele we were unable to find any such anastomoses between the vagus (or glossopharyngeus) and the sympathetic. In Dermophis there are two anastomoses between the ninth and tenth ganglia, one of which appears to be related to the sympathetic anastomosis above mentioned, that is, this anastomosis may be partly or wholly from the vagus ganglion, instead of from the glossopharyngeal.

In the larva of Ichthyophis (fig. 35) the IX–X nerve roots are indistinguishably commingled as they pass through the cranial wall. A small anterior ventral vagus ganglion, situated at the point where the two nerves diverge, is distinct from the more elongate dorsal glossopharyngeal ganglion. Both ganglia are apparently visceral sensory exclusively. On the vagus trunk is a third ganglion, evidently corresponding to the trunk vagal ganglion in Herpele. In Ichthyophis, however, there are two

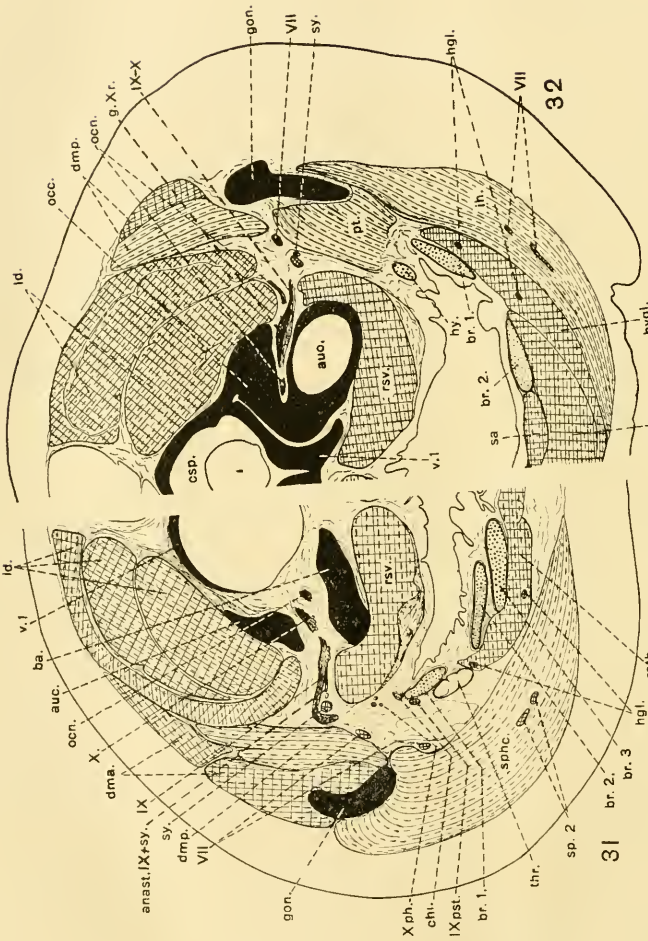
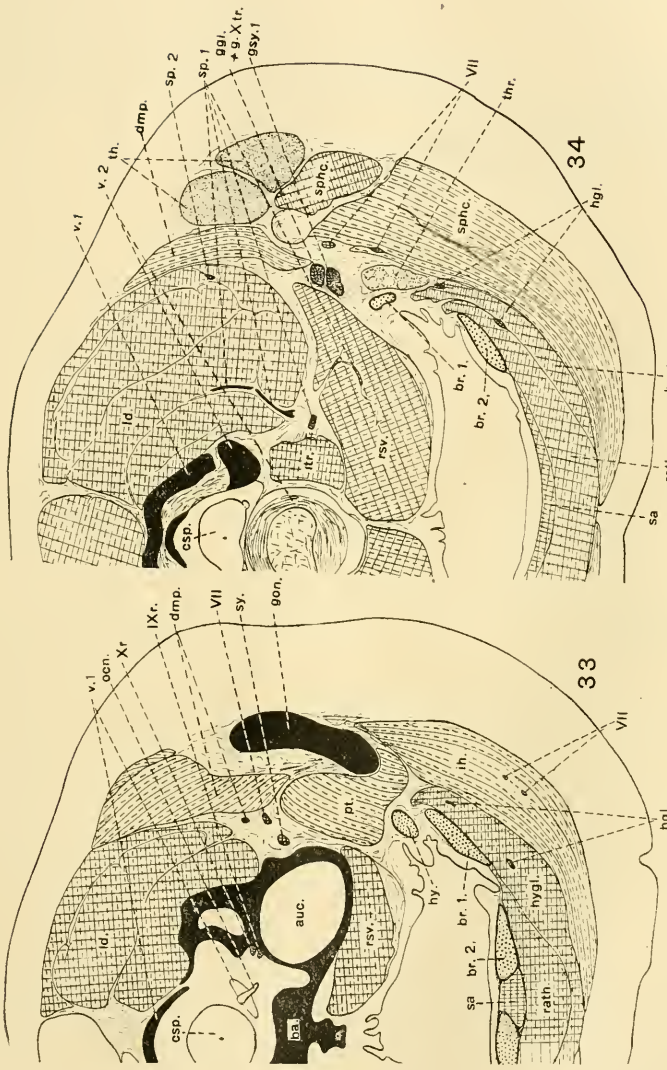


Fig. 31 Cross-section of the head, right half, through the origin of the IX–X cranial nerves of Dermophis. × 7.5.

Fig. 32 Similar section, left half, of Herpele; section 293. × 12.5.



Figs. 33 and 34 Cross-sections of the head, left half of Herpele; fig. 33, through the root of the occipital nerve; fig. 34, through the first cervical sympathetic ganglion; sections 287 and 319. × 12.5.

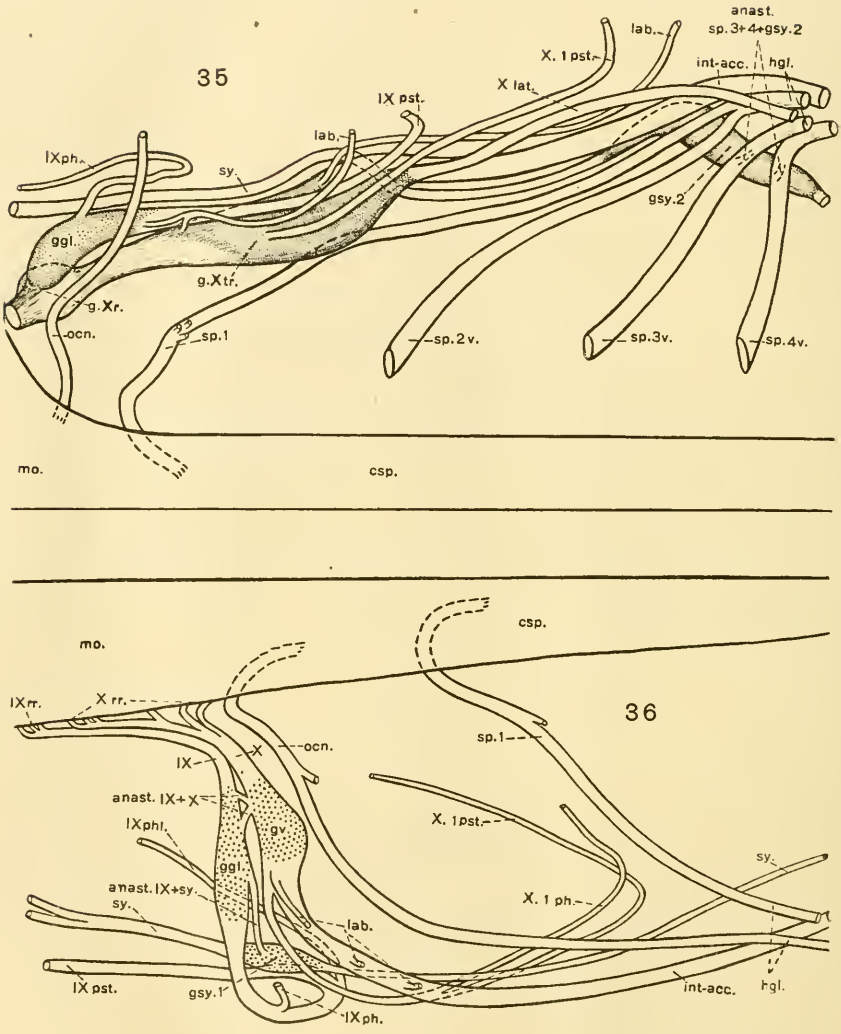


Fig. 35 A projection upon the horizontal plane of the ninth and tenth cranial nerves, principal rami and ganglia, the main trunks of the occipital and first spinal nerves, and the ventral rami of the second, third and fourth spinal nerves, together with the sympathetic trunk and ganglion, of a late larval stage of Ichthyophis glutinosus. × 34.

Fig. 36 A similar projection of the ninth and tenth cranial nerves, the occipital and first spinal nerves, together with the sympathetic trunk and first cervical sympathetic ganglion, of Dermophis mexicanus. × 14.

distinct kinds of cells in this latter ganglion; large cells situated mostly dorsally along the tract of the lateralis fibers which run through the ganglion; small and medium cells which we interpret as visceral sensory or sympathetic in character. In the adult Ichthyophis the ganglia and their relations are essentially as in the larva, lacking, however, the lateralis elements. As stated by Wiedersheim, there is an anastomosis between the vagus and the sympathetic in the adult Ichthyophis.

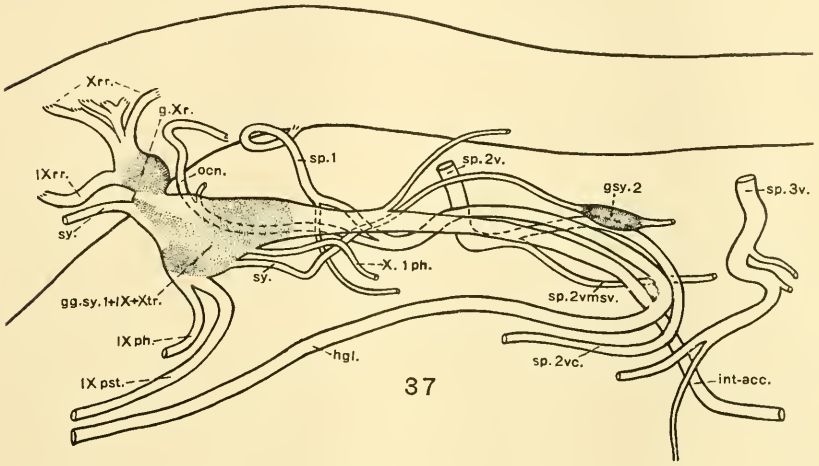


Fig. 37 A projection upon the sagittal plane of the ninth and tenth cranial nerves, the occipital and first spinal nerves, and the ventral rami of the second and third spinal nerves, together with the sympathetic trunk and ganglia, of Geotrypetes petersii. × 27.5.

In Geotrypetes the IX–X trunk passes out laterally, the fibers of the vagus through a rather large root ganglion, until the sympathetic trunk is reached, along whose dorsal border it runs posteriorly (fig. 37). Soon ganglion cells appear between the two trunks and shortly thereafter both are merged in a common ganglion. This ganglion evidently represents a fusion of a sympathetic, a glossopharyngeal, and a vagal trunk ganglion.

In Caecilia the roots of the IX–X nerves are distinct, separated by connective tissue as far out as the root ganglion of the vagus. The latter has an unusual situation, far out laterally

over the sympathetic trunk, close to the glossopharyngeal ganglion. A large trunk ganglion occurs on the right side of the single specimen examined, on the left side it is fused with the root ganglion. In Caecilia the second branchial nerve (*X.1*) arises from the vagal root ganglion, suggesting that in all the caecilians the root ganglion of the vagus is merely the ganglion of a branchial nerve.

Waldschmidt states that in Siphonops the vagus ganglion is united with a sympathetic ganglion. Marcus figures in the embryo of Hypogeophis three ganglia in the IX–X complex, distinct from the ganglia of the sympathetic trunk.

2. The glossopharyngeal or first branchial nerve

From the posterior end of the glosspharyngeal ganglion a nerve is given off which runs for a short distance posteriorly along the dorsolateral border of the sympathetic trunk, then curving ventrally around the lateral border of the latter and passing posteriorly as far as the level of the thyreoid gland, there divides, one branch going anteriorly medial to the thyreoid and then along the dorsal wall of the pharynx, ramus pharyngeus IX (fig. 44, *IXph.*). The second branch divides and sends one division anteriorly dorsal to the thyreoid gland along the border of the first branchial arch, later shifting dorsally to pass through the ceratohyoideus internus muscle, which it innervates, to the ventral wall of the mouth, passing along the inner border of the hyoid cartilage and into the base of the tongue, ramus lingualis IX (r. posttrematicus IX) (fig. 44, *IXpst.*).

In Dermophis the trunk of the ninth nerve is throughout distinct from the vagus. In its distribution and branching it is similar to the glossopharyngeal of Herpele.

From the ventral border of the ganglion, which in Geotrypetes is formed by a fusion of the glossopharyngeal, trunk vagus and sympathetic ganglia, there are successively given off two branchial nerves: 1) glossopharyngeal (fig. 37, *IXph., IXpst.*); 2) first vagal or second branchial (*Xph.*).

In the larva of Ichthyophis (fig. 35) the ninth nerve separates from the vagal root just as their respective ganglia are reached. From the ninth ganglion a small ramus pharyngeus (*IXph.*) is given off laterally, and from its posterior end a ramus posttrematicus (*IXpst.*) passes out. The sympathetic trunk is very closely associated with the glossopharyngeal, but an anastomosis was not with certainty detected. In the adult the relations are much the same.

3. The second branchial nerve

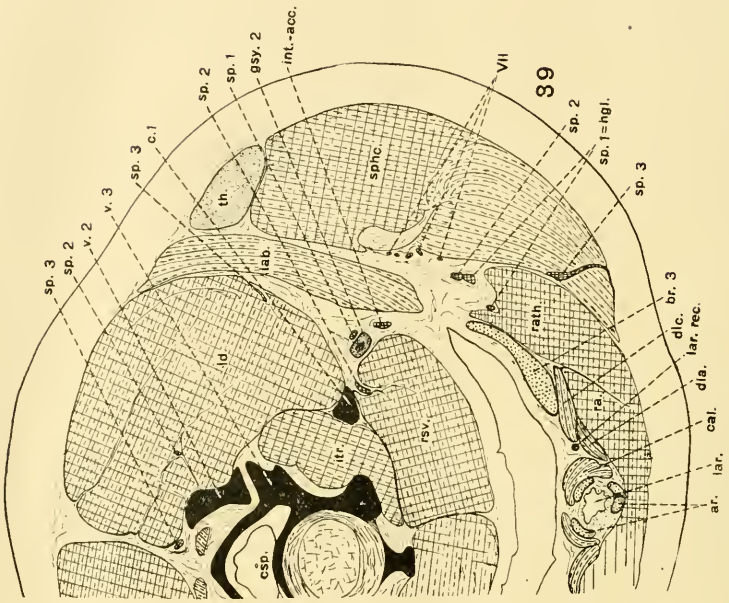
Arising in common with the first branchial nerve (fig. 38, *IX + X*), or more often from the trunk ganglion of the vagus, in Herpele, is a nerve which runs posteriorly at the dorsal border of the thyreoid gland, then, curving ventrally around the posterior border of the same gland, turns anteriorly to run along the lateral border of the second branchial cartilage, farther anteriorly along the pharyngeal epithelium between the second and third branchial arches, ramus posttrematicus X.1. Before curving around the thyreoid it may give off a ramus pharyngeus to the dorsal pharynx, or the latter may arise separately from the main tenth trunk. From the main ramus, just as it is curving anteriorly, a posteriorly directed branch is given off, passing to the lateral wall of the pharynx, possibly a remnant of a third branchial nerve. The second branchial nerve is exclusively sensory (fig. 44, *X.1ph.*, *X.1pst.*, *X.1php.*). Both the first and second branchial nerves in Herpele are in some parts much atrophied.

In Dermophis, as seen in figure 36, the second branchial nerve is not greatly different from the condition in Herpele.

As mentioned in a previous section, the second branchial nerve in Geotrypetes arises from the IX-X-sympathetic ganglion in the form of a ramus pharyngeus (fig. 37, *X.1ph.*).

In the larva of Ichthyophis a second branchial nerve arises from the dorsal border of the vagal trunk ganglion (fig. 35, *X.1pst.*).

In Caecilia, as stated in a preceding section, the second branchial nerve arises from the root ganglion of the vagus.


39
sp. 3
sp. 2
v. 2
v. 3
sp. 3
c. 1
sp. 2
sp. 1
gsy. 2
int.-acc.
VII
sp. 2
sp. 1=hgl.
sp. 3
br. 3
dlc.
lar. rec.
dla.
cal.
lar.
ar.
th.
sphc.
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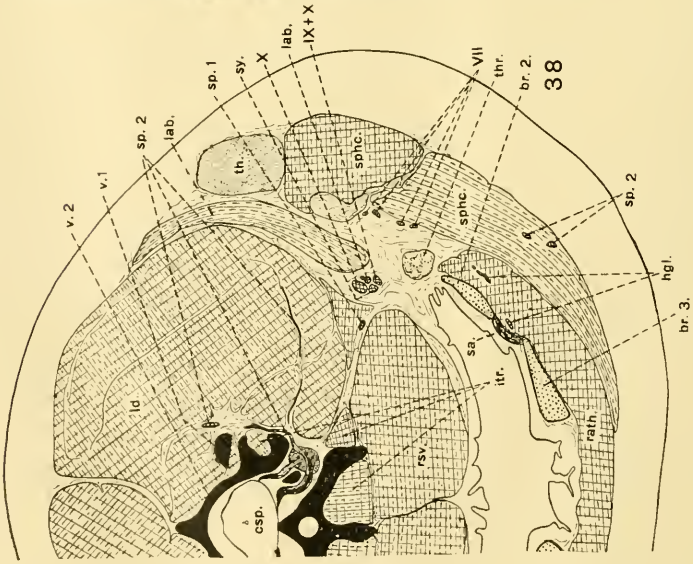
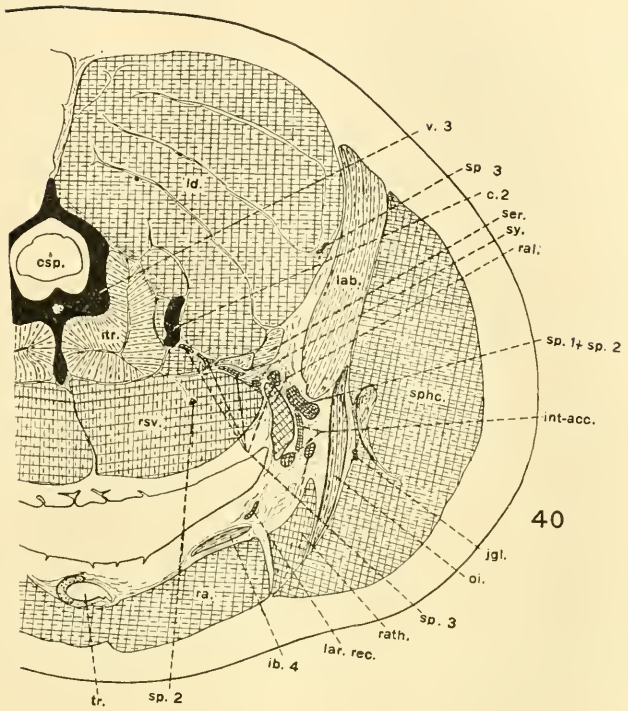

38
v. 2
v. 1
sp. 2
lab.
sp. 1
sy.
X
lab.
IX+X
VII
thr.
br. 2.
sp. 2
hgl.
br. 3.
th.
sphc.
sphc.
sa.
itr.
ld.
rsv.
csp.
rath.



Figs. 38 to 40 Cross-sections of the anterior trunk region, left half, of Herpele; sections 339, 378 and 403. × 12.5.

4. The ramus intestino-accessorius X

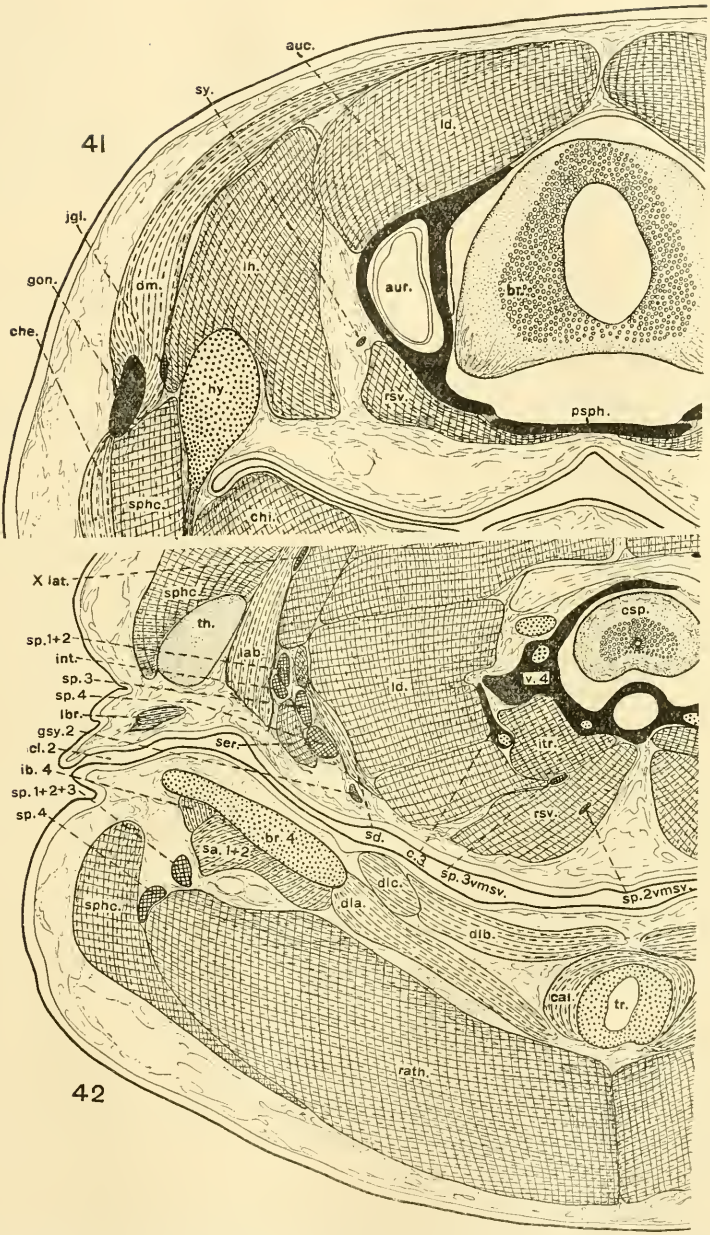
After giving off the branchial nerves, the continuation of the IX–X trunk consists of visceral sensory and visceral motor fibers. It runs directly posteriorly from the trunk ganglion, giving off two branches to the levator arcuum branchialium muscle (figs. 38, 44, *lab.*) At a level slightly posterior to the roots of the third spinal nerve the ramus intestino-accessorius curves ventrally around to the lateral border of the digestive tract (fig. 40, *int.-acc.*), dividing into an anterior laryngeus recurrens, of motor and sensory fibers, distributed to the interbranchialis 4 and laryngeal muscles and adjacent mucous epithelium (figs. 39, 40, 44, *lar.rec.*), a medial sensory branch that passes

posteriorly along the lateral surface of the trachea, and a posterior sensory ramus intestinalis which passes posteriorly at the lateral border of the digestive tract (fig. 44, *int.*).

The laryngeal muscles: In the late larval stage of Ichthyophis, according to Göppert ('94), whose statements the writer corroborates, there are five pairs of laryngeal muscles: 1) m. hyopharyngeus (dorsolaryngeus a), (fig. 42, *dla.*), originating on the fourth branchial arch in two parts, an anterior portion on the ventral side of the arch and a posterior on the dorsal end of the arch; the two parts of the muscle unite in an insertion on the medial raphe ventral to the trachea, a few anterior fibers attached to the ventral rectus series of muscles; 2) m. hyopharyngeus internus (dorsolaryngeus b) (*dlb*) originating on the dorsal border of the fourth branchial cartilage and posteriorly from connective tissue lateral to the pharynx, and meeting its fellow of the opposite side on the middle line between the trachea and the ventral wall of the pharynx, in part inserted on the lateral border of the trachea; 3) m. dilatator laryngis (dorsolaryngeus c), (*dlc.*), originating on the fourth branchial arch close to the origin of m. hyopharyngeus, and inserted on the lateral border of the arytenoid cartilage; 4) m. sphincter (constrictor aditus laryngis) (*cal.*); 5) m. laryngeus ventralis, inserted on the arytenoid cartilage and originating on the medial ventral line ventral to the larynx.

In Dermophis the arrangement is fundamentally as in Ichthyophis, except that the laryngeus ventralis is not sharply distinct from the sphincter. The hyopharyngeal and sphincter muscles are attached to the third (3rd + 4th) branchial cartilage.

In Herpele the hyopharyngeus internus muscle is absent. The following muscles attached to the larynx or in its immediate neighborhood and innervated by the ramus laryngeus recurrens X, occur (fig. 39): 1) m. constrictor aditus laryngis (sphincter) (*cal.*); 2) m. dorsolaryngeus (*c*) (dilatator aditus laryngis = dorsopharyngeus, Göppert) (*dlc.*), inserted on the arytenoid cartilage; 3) m. dorsolaryngeus (a) (hyopharyngeus, Göppert, in part = dorsotrachealis, auct.) (*dla.*), inserted in a tendon connected with the corresponding muscle of the opposite side beneath the



Figs. 41 and 42 Cross-sections of a late larval stage of Ichthyophis, right half; fig. 41 through the posterior part of the ear capsule, and the levator hyoidei muscle; fig. 42 through the second gill-cleft and the laryngeal muscles. × 37.5

trachea; 4) m. interbranchialis 4 (hyotrachealis, auct.). This latter muscle is a small slip attached to the extreme posterior end of the third branchial cartilage, running thence posteriorly, medially and ventrally and inserted on the lateral border of the trachea (fig. 40. *ib.4*). In Dermophis this is apparently a posterior part of the hyopharyngeus of Göppert (dorsolaryngeus (a)).

5. General considerations on the glossopharyngeal and vagus nerves

As in other amphibia, the ninth and tenth nerves in the caecilians are closely associated, both in roots and in ganglia. It is possible, however, in some instances to distinguish sharply between them. It is then seen that the ninth nerve has the characteristic composition of visceral sensory and visceral motor fibers. A ramus pretrematicus IX, a very conspicuous nerve in the Urodela, with characteristic Jacobson's commissure connecting with the facial nerve, is absent. But if we rightly interpret Marcus' figures, a well differentiated ramus pretrematicus IX occurs in the larval stage (Hypogeophis). In the late larval stage of Ichthyophis the writers find no ramus pretrematicus IX. A 'ramus communicans' between the tenth (and ninth) and the seventh nerves, so characteristic of other amphibians is absent in the caecilians. A dorsal cutaneous branch (ramus auricularis X of Urodela and Anura) of the vagus does not exist. This is somewhat remarkable, as in other Amphibia it is a conspicuous nerve in the occipital region. Wiedersheim figures a dorsal branch of the vagus, but he evidently mistakes the occipital nerve for this. The absence of limbs is correlated with a lack of the trapezius and omo-arcualis muscles with their innervation from the ramus intestino-accessorius X. The term intestino-accessorius is used as corresponding to the nerve of that name in the Urodela, but, with the absence of limbs and the trapezius group of muscles, plainly most if not all of the accessory elements are wanting.

The IX–X group of nerves is simplified in the adult caecilians by the absence of the lateral line contingent of the larval stages.

According to Marcus, there are four lateral-line nerves in the trunk of the larva; one dorsal, two medial, and one ventral. They are completely absent in the adult. As will be seen later, the ramus lateralis profundus of Marcus is a motor branch of a spinal nerve. In the late larva of Ichthyophis there is a single lateral-line nerve supplying a dorsal series of neuromasts (figs. 35, 43, *X lat.*). The occurrence of a trunk ganglion on the vagus nerve introduces a feature entirely foreign to the Urodela and Anura. Willard ('15) reports the occurrence of a trunk ganglion on the tenth nerve in Anolis, and an absence of a dorsal cutaneous branch. Only two pairs of distinct branchial nerves are represented in the adult caecilians, a condition not unexpected considering the rudimentary condition of the branchial arches.

THE OCCIPITAL NERVE

A distinct nerve between the vagus and the hypoglossal nerves is uncommon in the Urodela and Anura. Fürbringer ('97) finds an occipital nerve in Cryptobranchus japonicus. Druner (1901, 1904) reports the same in C. alleghaniensis and indications of it in Salamandra and Triton. Wiedersheim ('79) figures, in Siphonops, a dorsal branch of the vagus nerve, which is doubtless the occipital. Marcus ('10) describes an occipital nerve in the larva of Hypogeophis.

In Herpele the occipital nerve arises from the ventrolateral border of the neural tube near the border-line between brain and spinal cord. It passes anteriorly until the anterior border of the first vertebra is reached, where it turns abruptly latero-ventrally and then posteriorly around the anterior border of the vertebra and the occipital condyle, in the space between the latter and the posterior wall of the ear capsule (figs. 32, 33, *ocn.*). The nerve runs out parallel with the IX–X roots, but separated from them by a blood-vessel, and passes across and through the posterior border of the vagus root ganglion in such a way as to give the appearance of being a part of the ninth-tenth complex. Leaving the root ganglion, it gives a small twig to the anterior part of the rectus subvertebralis muscle and then passes into the dorsal trunk musculature, anastomosing with a dorsal branch of the first spinal nerve.

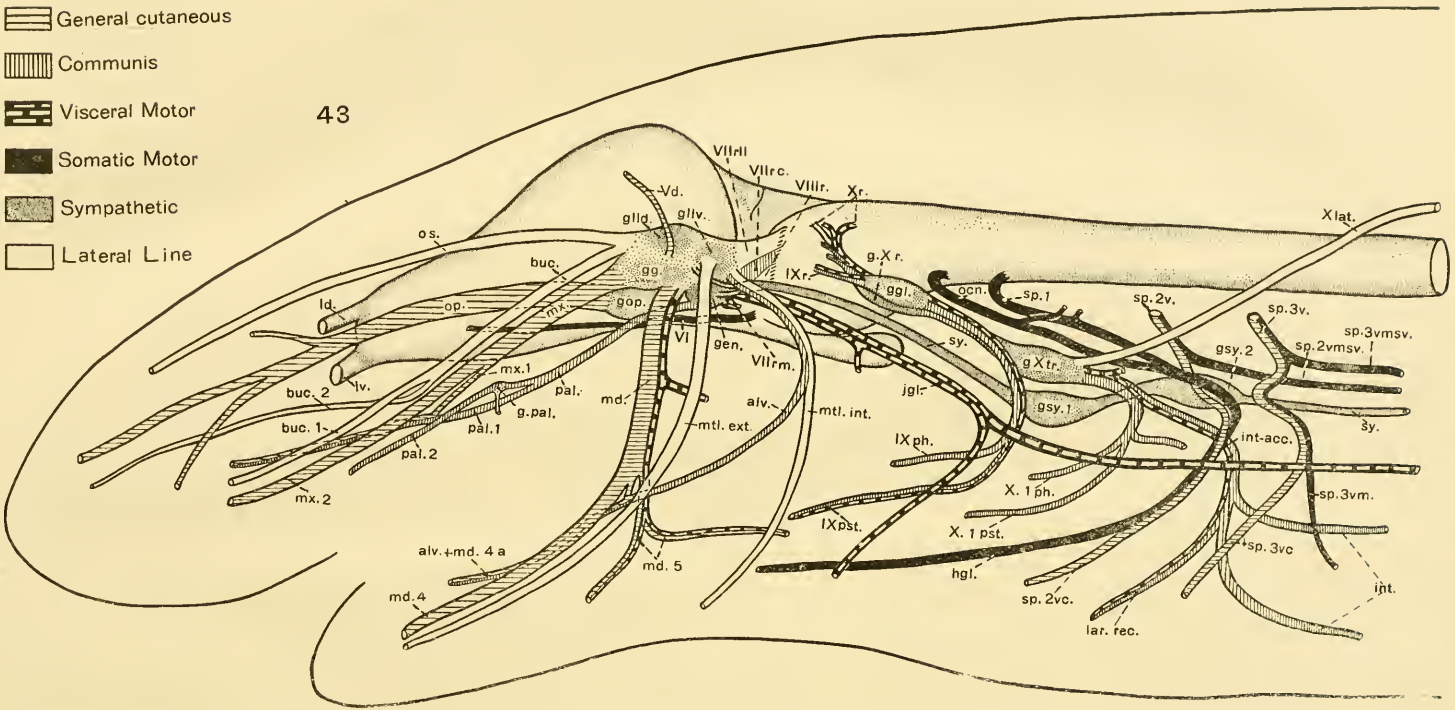


Fig. 43 A diagrammatic projection upon the sagittal plane of the cranial nerves of a larval caecilian, constructed by superimposing upon the adult condition in Herpele the lateral line system of a late larval stage of Ichthyophis. Of the eye-muscle nerves only the functionally developed abducens is shown.

In Ichthyophis the origin and course of the occipital nerve are much as in Herpele (fig. 35) but it nowhere comes in contact with the vague root ganglion. In Geotrypetes, Dermophis and Caecilia (figs. 37, 36) the occipital nerve contributes an important element to the hypoglossal nerve.

In the occipital nerve we see represented a ventral division of a spinal nerve. It possesses two parts: a ventral branch to the hypaxonic musculature, with sometimes a more specialized contribution to the hypoglossal area, and a dorsal branch to the dorsal trunk musculature. Of the forms studied, only in Herpele does the occipital pass through the vagus ganglion. We may therefore infer that there is no anastomosis between the occipital and the vagus in any caecilian.

THE FIRST SPINAL NERVE

This nerve arises from the central nervous system in a manner similar to the preceding nerve, and passes out through a canal in the first vertebra, the characteristic exit of the first spinal nerve in Amphibia. Soon after emerging it divides into dorsal and ventral divisions, (fig. 34, *sp.1*) the former of which goes to the dorsal trunk muscles, and the latter, after sending off a small branch to the rectus subvertebralis muscle, passes posteriorly and laterally in the interval between the lateral and medial portions of this muscle (fig. 38), and approaches the main trunks of the sympathetic and vagus nerves, parallel to which it runs for some distance. Then it gradually passes, dorsal to the sympathetic, around to the lateral border of the second sympathetic ganglion (fig. 39), a short distance posterior to which it fuses with a cutaneous branch of the second spinal nerve (fig. 40, *sp.1* + *sp.2*) which has already passed through the sympathetic ganglion. The nerve trunk thus formed turns ventrally and anteriorly in the space between the levator arcuum branchialium muscle and the extreme anterior end of the lateral division of the rectus abdominis muscle, grazing the lateral border of the ramus intestino-accessorius X, as the latter curves ventrally from its anterior dorsal position (fig. 40). This trunk, formed by these

branches of the first and second spinal nerves, runs anteroventrally, slightly lateral to the wall of the pharynx, just ventral to the ventral border of the levator arcuum branchialium muscle. At about the level of the exit of the third spinal nerve the cutaneous component of this first and second spinal nerve derivative leaves as a dorsal branch (fig. 39, *sp.2*) which soon curves ventrally and laterally, and descends through the interval between the sphincter colli muscle laterally and the rectus abdominis muscle medially, finally passing through the former muscle (fig. 38) to be distributed laterally and ventrally to the skin in the posterior head and anterior trunk region.

The remainiug motor constituent forms the nervus hypoglossus (fig. 39, *sp.1* = *hgl.*). Just before and shortly after the separation of the cutaneous element small motor branches are given off which run to the ventral portion of the rectus abdominis musculature at the level of the fourth spinal nerve. The main trunk of the hypoglossus passes anteriorly at the lateral border of the thoracicohyoideus division of the ventral rectus abdominis muscle. It enters the latter muscle (fig. 38), gives off some small branches and then divides into a smaller dorsolateral and a larger ventromedial division, the former supplying the hypoglossus and the lateral portion of the geniohyoideus muscle and the latter the deeper medial portion of the thoracicohyoideus muscle and the geniohyoideus also (figs. 34–32, 25–20).

In Herpele the hypoglossus is thus formed from the first spinal nerve only. This is the characteristic origin of the nerve in the Amphibia. In Amphiuma the senior writer ('08) found in the hypoglossus only the first spinal nerve represented, Druner and others to the contrary. In Siren, as in Herpele, there is a union of branches of the first and second spinal nerves, but as motor and sensory branches are given off from the combination before the hypoglossus proper is reached, it is probable that only first spinal fibers remain in it. In Spelerpes Bowers ('00) believed the hypoglossus to be derived from the second spinal nerve. The writer finds that it is the first spinal nerve in Spelerpes that forms the hypoglossus. In Necturus, however, the hypoglossus is formed from the first and second spinal nerves.

In Geotrypetes, Dermophis and Caecilia an important branch of the occipital nerve combines with a branch of the first spinal nerve to form the hypoglossal nerve.

According to Fischer, both the first and second spinal nerves in Siphonops enter the sympathetic ganglion, and the hypoglossus emerges from the latter. Wiedersheim states that in Ichthyophis the hypoglossus is formed by the union of a branch of the first spinal nerve, which has passed through the sympathetic ganglion, with a ventral branch of the second spinal nerve, the latter not passing through the ganglion. The writers find that in the larva and adult of Ichthyophis neither the first nor the second spinal nerve passes through the sympathetic ganglion. Waldschmidt designates the second spinal nerve in Siphonops as the first, the true first spinal nerve becoming his hypoglossus. He finds no fusion with the second spinal nerve. Marcus sees in Hypogeophis a union of the first and second spinal nerves to form the hypoglossus. In the larva of Ichthyophis ventral branches of the first, second, and third spinal nerves unite in a hypobranchial nerve, from which, after turning ventrally and anteriorly, there are given off sensory and possibly motor branches, leaving the hypoglossus anteriorly.

THE SECOND SPINAL NERVE

The second spinal nerve has the full complement of a typical spinal nerve, with dorsal and ventral roots. Three main rami pass out of the ganglion: 1) a dorsal of mixed constitution, passing dorsally around close to the lateral border of the vertebra, thence up through the dorsal muscles near the middle line, to be distributed to the dorsal skin and the dorsal trunk musculature (fig. 44, *sp.2d.*); 2) a lateral cutaneous branch passes laterally out through the ventral part of the dorsal musculature in an almost horizontal position until it reaches the lateral portion of the musculature, where it divides into anterior and posterior branches distributed to the skin (*sp.2l.*); 3) a ventral branch of mixed composition directed laterally and posteriorly out through the notch formed by the tubercular and capitular processes of

the first rib and thence, in the interval between the dorsal and subvertebral trunk muscles, to the vicinity of the sympathetic trunk (*sp.2v.*). On the way it divides into *a*) a motor branch soon entering the rectus subvertebralis muscle and running through it as far as the series of sections continues (sp. *2vmsv.*), gradually diminishing in size as it gives off fibers to the muscle; *b*) a sensory branch, containing a few motor fibers (*sp.2vc.*). This latter branch passes through the second sympathetic ganglion, leaving the motor fibers within the ganglion, and, on emerging, runs laterally and ventrally to unite with the first spinal nerve as noted in the preceding section. Farther anteriorly from this union the sensory cutaneous fibers (second spinal nerve) separate from the motor fibers (first spinal nerve) and are distributed laterally and ventrally to the skin of the posterior head and anterior trunk regions. The small group of motor fibers, introduced into the sympathetic ganglion along with the cutaneous branch of the second spinal nerve, divides within the ganglion. The two minute branches thus formed pass out at the extreme posterior end of the ganglion, one of them uniting with a small branch of the third spinal nerve. With a small group of fibers of the latter it forms a small nerve that fuses with the other branch which arose from the division within the ganglion. The resulting nerve (fig. 44, *sp.2+3vm.*) passes posteriorly to innervate the muscle described by Wiedersheim as the 'serratus.'

THE THIRD SPINAL NERVE

The third spinal nerve conforms very closely to the plan of the second. A dorsal and a lateral ramus have a composition and a distribution similar to the corresponding branches of the second spinal nerve. The ventral ramus divides into: *a*) a motor branch which runs some distance at the lateral border of the rectus subvertebralis musculature, then enters the latter to continue a course similar to the corresponding branch of the second spinal; *b*) a cutaneous division which, like the corresponding branch of the second spinal, contains motor fibers. This cutaneous nerve passes out posterolaterally through the ventral

part of the dorsal musculature, and, turning around the posterior border of the tendon of origin of the serratus muscle, runs abruptly anteriorly and laterally until it reaches the posterior end of the second sympathetic ganglion. Here it receives a small group of motor fibers from the second spinal nerve, as noted above, and then turns abruptly posterolaterally and ventrally. A short distance posterior to the second sympathetic ganglion it gives off the second spinal fibers with some others, which fuse with another second spinal twig, as previously noticed, to form a nerve supplying the serratus muscle (*sp.2 + 3vm.*). The remaining motor fibers form a larger nerve that supplies the lateral portion of the rectus abdominis musculature (sp. *3vm.*). The main ramus, now wholly sensory (*sp.3vc.*), curves anteriorly around to the ventral side of the body and passes out between the ventral rectus abdominis and sphincter colli muscles, and finally through the latter to be distributed to the ventral and lateral skin somewhat posterior to the corresponding branch of the second spinal nerve.

Fischer represents four main branches of the third spinal nerve in Siphonops: 1) a communicating branch with the sympathetic ganglion; 2) an anteroventrally directed branch, which is plainly the sensory branch described above in Herpele (*sp.3vc.*); 3) a ventral posterior branch, which corresponds to the motor branch supplying the lateral rectus abdominis musculature in Herpele (*sp.3vm.*); 4) a so-called lateralis, which is beyond question the motor branch in Herpele that supplies the rectus subvertebralis musculature (*sp.3vmsv.*). Wiedersheim recognizes, in both the second and third spinal nerves of Ichthyophis, a large motor branch to the rectus subvertebralis muscles, which condition the writer finds in the larval stage. Marcus finds a similar branch of the third spinal in Hypogeophis, but designates it as a ramus lateralis profundus. In Geotrypetes the writer finds corresponding branches on the first three spinal nerves.

THE FOURTH, FIFTH, AND SIXTH SPINAL NERVES

Beginning with the fourth spinal nerve, there are marked changes in distribution. The main ventral ramus lacks any important branch to the rectus subvertebralis musculature, and, after emerging from the axial trunk muscles, gives off a cutaneous branch which passes dorsolaterally out over the dorsal border of the sphincter colli muscle to the skin mediolaterally (*vcl.*).

THE SYMPATHETIC CHAIN

The main sympathetic trunk in Herpele begins anteriorly by the union of two branches which arise, one from the lateral border of the posterior tip of the Gasserian ganglion and the other from the ventrolateral border of the geniculate ganglion (fig. 27, *sy.V*, *sy.VII*). The common trunk formed by these two nerves soon becomes associated with the hyomandibular trunk of the facialis, and by most observers has been considered as a part of the latter (fig. 29, *sy.*, *VII;* also fig. 44,). In some places the sympathetic and the facialis are bound together in one trunk, but the non-medullated character of the fibers of the sympathetic makes its recognition easy. The sympathetic trunk accompanies the hyomandibular (or ramus jugularis, more posteriorly) in its course posteriorly around the lateral border of the ear capsule. At the level of the exit of the IX–X nerves, the sympathetic has separated from the jugularis (fig. 32) and approached very closely to the IX–X trunk, running parallel with and along the ventral border of the latter to a point slightly posterior to the trunk ganglion of the vagus (figs. 34, 38). Thence the vagus drops ventrally around the lateral border of the sympathetic and is thenceforth ventral and lateral to the latter (figs. 39, 40). The two trunks continue in this parallel course until the vagus curves ventrally to its distribution into its chief terminal branches. At the level of the posterior third of the trunk ganglion of the vagus, the sympathetic enters the first great sympathetic ganglion (figs. 34, 44, *gsy.1*). Farther posteriorly, between the levels of the second and third spinal nerves, is a second still larger sympathetic ganglion (figs. 39,

44, *gsy.2*). From the posterior end of this second ganglion the sympathetic is continued as a small nerve which ends by anastomosing with the ventral ramus of the fourth spinal nerve. As the fibers of the first branchial nerve, or the common trunk of the first and second branchial nerves, leave the IX–X trunks they run along the dorsal border of the first sympathetic ganglion and may be imbedded in its substance. It is impossible to determine whether fibers in this way pass from the trunk of the vagus into the sympathetic or vice versa. As previously described, a cutaneous division of the ventral ramus of the second spinal nerve passes through the second sympathetic ganglion. Shortly before this cutaneous ramus reaches the ganglion, a non-myelinated bundle becomes distinguishable in the nerve, evidently the equivalent of a ramus communicans. With the cutaneous branch a small number of motor fibers enter the second sympathetic ganglion, to emerge separately from the ganglion as already described. With the extreme posterior end of the second sympathetic ganglion the cutaneous branch of the ventral ramus of the third spinal nerve comes into contact. There is possible an actual anastomosis with fibrous exchange. No non-myelinated constituent of the third nerve was found which could be interpreted as a ramus communicans. Beyond the second sympathetic ganglion the sympathetic chain is traceable only as a minute strand which terminates posteriorly in the fourth spinal nerve, as stated above.

In Herpele no anastomoses between the IX–X complex and the sympathetic occur unless the first and second branchial nerves, as described above, serve as a course along which such fibers pass. In Dermophis there is a large anastomosing branch between the glossopharyngeus (and possibly the vagus) and the sympathetic trunk (fig. 36, *anast.IX + sy.*). A similar branch is figured by Wiedersheim in Ichthyophis, by Fischer and by Waldschmidt in Siphonops. In the larva of Ichthyophis there is no very evident anastomosis between the sympathetic and the IX–X nerves, although there is contact in many places. In the adult of Ichthyophis, however, the writers confirm the statement of Wiedersheim. According to the figures of the latter author,

there are four or more sympathetic ganglia in Ichthyophis. In the larva of Ichthyophis the writers find only one sympathetic ganglion, corresponding to the second ganglion in Herpele (fig. 35). In the adult Ichthyophis there are two sympathetic ganglia, but the second ganglion may show constrictions, thus simulating the appearance of a chain of ganglia such as Wiedersheim represents. Fischer describes one large sympathetic ganglion in Siphonops with which the first and second spinal nerves anastomose. Waldschmidt figures three or four sympathetic ganglia in the same form. Marcus recognizes two sympathetic ganglia in Hypogeophis. He derives the ganglia of the sympathetic from the neural crest. In Dermophis there is no separate trunk ganglion on the vagus, but there are two ganglia on the sympathetic. Geotrypetes has two sympathetic ganglia, of which the anterior one is fused with the glossopharyngeal and vagal trunk ganglion. In the single specimen of Caecilia examined by the writers there are three sympathetic ganglia on the right side and four on the left.

In Siphonops Fischer states that the first and second spinal nerves pass into the single large sympathetic ganglion. In this same form, according to Waldschmidt, the first four spinal nerves anastomose with the sympathetic chain. Wiedersheim sees in Ichthyophis the first spinal nerve passing through the anterior sympathetic ganglion. In the larva and adult of Ichthyophis the writers find no anastomoses between the first and second spinal nerves and the sympathetic ganglia, but that there is a fibrous connection between the third and fourth spinal nerves and the second sympathetic ganglion. In Geotrypetes a ventral branch of the second spinal nerve comes into contact with the second sympathetic ganglion, but there is apparently no anastomosis (fig. 37).

According to Wiedersheim, the sympathetic chain arises anteriorly from the trigeminus in Ichthyophis. The writers find a similar origin in Geotrypetes, from the Gasserian ganglion. In Dermophis the sympathetic trunk begins by four connections, two each, with the Gasserian and geniculate ganglia. Marcus' ramus recurrens VII is the anterior part of the sympathetic chain connecting with the geniculate ganglion.

Despite the conflicting testimony of observers and the varying relations of the structural parts, the main cervical sympathetic chain in the caecilians may be said to consist typically of two ganglia connected anteriorly with the Gasserian and geniculate ganglia, and related by anastomoses with the IX–X complex and the anterior spinal nerves. The anterior sympathetic ganglion occurs usually at a level slightly posterior to the root of the occipital nerve. It may or may not be closely related to the trunk ganglion of the vagus. The second sympathetic ganglion is situated at a level between the roots of the second and third spinal nerves. Its frequent intimate relation to the second spinal nerve is to be regarded as largely incidental, although in effect a ramus communicans thus may be established.

GENERAL STATEMENTS

For one in search of primitive anatomical characters in the caecilians a study of the nervous system gives little comfort. The impression gained, rather, is that we are dealing here with highly specialized animals.

In the nervous system of the caecilians the amphibian type is overwhelmingly dominant, but with modifictions, these modifications not being in the direction of supposedly ancestral simplicity. The double condition of the olfactorius; the occurrence of two ganglia upon the trigeminus; the separation of a second branchial ganglion, as the writers are inclined to interpret the root ganglion upon the vagus, from the other vagal ganglia; these which may be interpreted as primitive characteristics the writers look upon as incidental exaggerations of conditions characteristic of amphibians in general.

The absence of structures characteristic of other amphibians is more striking than the presence of supposedly primitive characters. The mode of life of the caecilians necessitates the vestigial condition, or even absence of certain structures, as the eyes and associated parts and the limbs and related muscles. But quite unrelated to such modifications are the absence of 1) a ramus pretrematicus IX; 2), a Jacobson's commissure; 3) a 'ramus communicans' between glossopharyngeal and facial nerves; 4) a ramus auricularis X; 5) an anastomosis between the ramus ophthalmicus profundus V and the ramus palatinus VII.

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Fig. 44 A projection upon the sagittal plane of the cranial, occipital and anterior spinal nerves of Herpele ochrocephalum. The auditory nerve and ganglion are omitted. × 16.5.

50
100
150
200
250
300
350
400
450
500
550
600
General cutaneous
Communis
Visceral Motor
Somatic Motor
Sympathetic
Lateral Line
44

Resumido por el autor, E. H. Dusham.

Las glándulas productoras de cera en la cucaracha (Blatta germanica).

La hipodermis de los insectos ha sido descrita como formada por una sola capa de células. Una excepción se presenta en la pared dorsal del abdomen del macho de la cucaracha (Periplaneta orientalis), cuya hipodermis está formada por dos o tres capas de células. El estudio de las paredes del abdomen ha demostrado que en esta especie dicha estructura existe, no solo en las paredes dorsal y ventral del macho, sino también en las de la hembra. El autor ha encontrado también una estructura semejante en Blatta germanica. La capa superior está formada por células hipodérmicas semejantes a las que existen en otras regiones del cuerpo, en las que existen células hipodérmicas típicas. Las capas más profundas están formadas por células mucho mayores, con grandes núcleos vesiculares y citoplasma granuloso. Su aspecto irregular indica su naturaleza glandular, comprobada además por el hallazgo de canales pequeños que se extienden a través de la cuticula que recubre a dichas glándulas, la cual presenta pequeños poros en vista superficial, y por medio de los cuales la secreción sale a la superficie. El análisis de dicha secreción, recogida en la cuticula, ha demostrado que está formada de cera. En Blatta germanica estas glándulas se distinguen por primera vez despues de la primera muda del tegumento, en las ninfas de dos dias, en las cuales algunas de las células hipodérmicas que ocupan ciertas áreas, aumentan de tamaño y empujando a las células hipodérmicas adyacentes dan a la hipodermis el aspecto de una capa poliestratificada. La hipodermis de la cucaracha, por esta causa, no difiere de la de los otros insectos.

Translation by Dr. José Nonidez,
Columbia University.

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THE WAX GLANDS OF THE COCKROACH (BLATTA GERMANICA)

E. H. DUSHAM

Department of Entomology, Cornell University

ELEVEN FIGURES

INTRODUCTION

The ability to secrete wax in one form or another is widespread among insects, being reported for the Libellulidae of the Odonata; Notonectidae, Fulgoridae, Cercopidae, Psyllidae, Aphididae, Aleyrodidae, and Coccidae of the Hemiptera; Tortricidae and Papilionidae of the Lepidoptera; Coccinellidae and Curculionidae of the Coleoptera; and the Tenthredinidae and Apidae of the Hymenoptera.

That this function should also be found in cockroaches is not surprising, for they are waxy in appearance, feel waxy when handled, and when thrown into water or other fluids float readily as if buoyed up by some oily covering, even after the air has been forced out of the tracheae by pressure. In fact, one of the difficulties encountered in fixing material is to submerge the specimens in fixing fluids, especially when these are used cold. On the other hand, no such difficulty is experienced when hot fluids are used, the hot liquid evidently melting the wax, thus allowing the insects to sink.

Moreover, it is a well-known fact that the species in question is usually found in warm, damp places, especially in close proximity to sweating water pipes, its association with water pipes leading from the Croton Aqueduct in the vicinity of New York City earning for it the popular name of 'Croton bug.' Furthermore, references to the literature of this species show that it has been carried for long distances in water mains without drown-

ing. That they have been able to live in moist places and also withstand submerging would lead one to suppose that their bodies were protected by some oily or waxy secretion.

HISTORICAL

The study of the wax glands of the cockroach is bound up in a controversy as to the number of layers in the hypodermis of these insects. In insects in general the body wall is relatively simple, consisting of an outer chitinous covering or cuticula, the hypodermis consisting of a single layer of cells, and the basement membrane, and as such, Miall and Denny described it in their work on the cockroach, in which they stated that the hypodermis consisted of a single layer of flattened cells, resting on a basement mebrane, each cell corresponding to a polygonal area of the overlying cuticula.

In 1888, however, E. A. Minchin, while studying certain glands on the dorsal side of the cockroach, found that the hypodermis, at least on the dorsal side of the older stages of the male, consisted of two layers everywhere except in the intersegmental areas where only a single layer was present. In certain places he found that the cells of these layers had the appearance of giant cells with large nuclei, granular contents, and elongate processes. These he interpreted as ganglion cells. He found them scattered over each tergum, especially at the anterior portion of each tergite—that part which is overlapped by the preceding tergite—although they were also scattered throughout the region posterior to this part. Between these so-called ganglion cells he found the ordinary cells of the hypodermis. He therefore concluded that in the cockroach, at least, the hypodermis consisted of an upper layer of cells corresponding to the polygonal areas of the cuticula, and a lower very irregular layer, occasionally forming two layers, whose cells were modified to form nerve cells, and which were probably connected with setae where the terga were exposed.

In 1889, Mingazzini made a detailed study of this apparently double-layered hypodermis in order to verify Minchin's work,

examining the dorsal wall of the abdomen of males in different stages of development. He found that the hypodermis was not always made up of two layers of cells as Minchin described, but sometimes showed a single layer of cells, other times two or more layers. In the intersegmental areas, he found a single layer of cells as Minchin described. Where the muscle bundles were inserted he also found a single layer of cells, modified in the usual way to form the characteristic muscle attachments. In other places the hypodermis consisted of a single layer of cells, usually of small size, or of two layers, the upper layer consisting of small cells, the lower one of much larger cells, or of several layers of cells arranged without order. Except for an occasional large cell at the surface among the smaller cells where a single layer of cells was present, the majority of the larger cells were below the smaller ones.

He concluded that these cells were not to be considered as nerve cells, but as cells of an epithelial nature, derived from the upper layer of the hypodermis, but which had undergone enlargement and were no longer of use in the secretion of the cuticula. He was uncertain whether these large cells were hair-forming cells or gland cells, but because of the structure of the nucleus and protoplasm he favored the glandular view, the insects probably secreting an oily substance. He, therefore, concluded that the hypodermis of the cockroach did not differ from that of other insects by the nature of the cells which composed it, but only because, as the insects increase in size, many hypodermal cells become specialized for a particular function, increased in size, took on a branching form, and were carried below the regular hypodermis, thus forming an apparently double-layered hypodermis.

In 1909, Berlese, in commenting on this peculiar structure in the cockroach, stated that if the smaller cells were not merely infiltrated amoebocytes, they were to be regarded as a proliferation of the hypodermis, and that possibly the larger cells had assumed another function. At least, he was sure that the condition was unique among insects and was still an open question.

LOCATION AND STRUCTURE OF THE GLANDS

At the suggestion of Dr. W A. Riley, under whose direction this work was carried out, the writer undertook to settle this question. While the preceding investigations were made on Periplaneta orientalis, yet a study of Blatta germanica showed that similar conditions were present, and because of the abundance of the latter, this species was used almost entirely in the following work.

In order to show the general distribution of the glands, the abdomen was cut from the anterior part of the body, and a slit through the chitin was made all around its margin, thus separating the dorsal from the ventral wall. From these respective parts the fat and muscle were removed as carefully as possible so as not to injure the delicate hypodermis. It was found more advisable to fix the entire abdomen before making the incision around the edge, because the greater rigidity after fixation facilitated cutting, and prevented the parts from curling, as when fresh material was cut. Both dorsal and ventral abdominal walls were then stained with Grenacher's borax carmine and Delafield's haematoxylin, and mounted in balsam. Better results were obtained with the latter stain. The gland cells are so small that they can hardly be distinguished from the normal hypodermis except for the larger size of their nuclei, and these were rendered more conspicuous by using Delafield's haematoxylin and destaining with acid alcohol until the nuclei stood out prominently, while the surrounding cytoplasm of the cells was but faintly tinged. The nuclei are even better accentuated by dipping the material in alkaline alcohol after destaining in acid alcohol, as they take on a deep blue coloration after such treatment.

As the glands are most prominent at the anterior portion of each segment, and as this portion is covered by the posterior part of the preceding segment, some difficulty was encountered in making out their distribution. However, by injecting the abdomen with killing fluid by means of a hypodermic needle so that the intersegmental membranes were stretched taut on a plane with the rest of the tergite or sternite, all the glandular areas were

exposed. Bouin's fluid proved the most practical for this purpose. When the parts were fully extended, they were plunged into hot killing fluid and the pressure maintained until the hot killing fluid without and the killing fluid within had so fixed the material that the parts would remain extended when the pressure from the hypodermic needle was released.

For histological study the material was fixed in Fleming's (strong fluid), Gilson's, Zenker's, Bouin's, and Dietrich's fluids. Best cytological results were obtained with Fleming's, although Dietrich's and Bouin's fluids gave almost as good results. With both of the latter more rapid penetration certainly was obtained. Gilson's and Zenker's fluids gave almost similar results.

At first, material was imbedded entirely in paraffin, but when sections were cut, it was found that the chitin was very brittle and broke very readily, often catching on the edge of the knife and tearing gaps through the entire section. Because of this, sections were so mutilated that they were often useless, especially as the parts desired for study were immediately under the chitin. To obviate this difficulty several methods were tried either to soften the chitin or else to hold the parts in place so that they would maintain their normal position as nearly as possible. Metalinkoff's method, by which the object was first imbedded in paraffin in the usual way, and then all the paraffin scraped from the surface of the chitin, the object immersed in Eau de Javelle for twenty-four hours, and re-imbedded, did not give good results. But as this method was tried in only a few cases perhaps repeated trials would have proved more successful. Bedau's 'Seifenspiritus' method gave no results, although followed as exactly as possible.

The combined paraffin and celloidin method was also used. Three different modifications of this method were employed—Gilson's rapid process, Apathy's method, and Hoffman's method. All three gave good results, and longitudinal sections through the entire abdomen, 5 micra thick, were easily obtained.

Cross and longitudinal sections of the entire abdomen were cut from 4 to 10 micra in thickness. These were fixed to slides by means of the Mayer albumen-and-water method. Instead,

however, of first rubbing a drop of albumen on the slide and then adding water, three drops of the albumen were added to a watch-glass of distilled water, thoroughly mixed with it, and a layer of this was applied to the slide with a pipette. Sections were then placed on this and flattened by means of heat. By this method the least amount of albumen necessary remains on the slide, but always enough to hold the sections firmly in place.

Sections were stained with Heidenhain's iron haematoxylin and Delafield's haematoxylin with eosin as a counterstain. Better cytological results were obtained with the former, especially after fixation with Fleming's fluid, although good results were also obtained after fixation with Bouin's fluid. However, for general histological work, Delafield's haematoxylin and eosin gave very good preparations.

Prepared mounts of the entire dorsal and ventral walls, supplemented by cross and longitudinal sections of the entire abdomen, showed that these modified hypodermal cells were present in each segment, not only on the dorsal side of the male, but on the ventral side also, and in the female as well as the male (figs. 1, 2, 3, and 4). Except in the intersegmental membranes, they are scattered over each tergite and sternite, being extremely abundant at the fore part of each segment laterad of the median line, especially that part of the segment which is overlapped by the preceding segment. Posterior to this region, i.e., where the tergite or sternite is not covered by the preceding segment, these modified cells are more scattered, occurring here and there among the normal hypodermal cells.

Longitudinal sections show that in the intersegmental membranes there is but a single layer of unmodified hypodermal cells (fig. 8, *i*, and fig. 9, *d*). The nuclei of these cells are somewhat elongate and flattened, with their long axes parallel to the surface of the overlying cuticula; they are surrounded by but a small amount of cytoplasm, so slight in cases that it appears thread-like with the nuclei bulging out here and there. The nuclei themselves are very deeply stained and are fairly regularly arranged.

At the anterior end of each segment—that part which is overlapped by the preceding segment—the modified hypodermal cells are especially well developed, often producing the appearance of two or more layers of cells, no doubt due to the crowding of the individual cells, with the consequent displacement of their nuclei (figs. 8 and 9). This is best seen on the ventral side of the male where the cells are closely packed together so that there seems to be no order to their arrangement. In such places the cells also appear of different sizes, no doubt due to the fact that a single section did not pass through the middle of each cell. In other places they are but a single layer in thickness.

These modified hypodermal cells possess large vesicular nuclei with relatively thick nuclear walls, and provided with a nucleolus and many deeply stained chromatin granules (fig. 10). Their cytoplasm is also coarsely granular in appearance, and contains several vacuoles. As a rule, they are cuboidal or columnar, except where crowding together has caused them to assume various forms. Scattered here and there between these cells and the overlying cuticula, or intercalated somewhat between them at their upper surface, are the non-glandular hypodermal cells with nuclei similar to those in the intersegmental areas.

Posterior to that portion of the segment which is overlapped by the preceding segment, the normal hypodermal cells are in the majority, while here and there scattered among them are large modified cells similar to those occurring at the anterior end of the segment. Where such cells occur, the ordinary hypodermal cells are absent or else are somewhat crowded together.

From the foregoing observations, therefore, it will be seen that the body wall of the cockroach does not differ materially from that of other insects, being composed of a cuticula, a hypodermis consisting of a single layer of cells, and a basement membrane. However, many of the hypodermal cells have become specialized, increasing in size, so that in places, particularly on the ventral side of the male, they present the appearance of two or more layers. The cytoplasm of these cells has become strongly granular while the nuclei have become large and vesicular. Their entire structure, therefore, indicates that they are secretory in

nature, and are therefore gland cells. Their secretion passes to the exterior through very minute pores through the chitin (fig. 7). These are evident only in very thin sections, cut exactly perpendicular to the surface of the cuticula. Otherwise it is almost impossible to make them out. With favorable sections they show as a fine streaking of the cuticula (fig. 10, *p*).

In portions of the cuticula stained black with osmic acid the pores also show as minute white dots under an oil-immerson lens. The secretion passes through these pores to the exterior and without doubt spreads in a fine film over the outer surface of the cuticula.

NATURE OF THE SECRETION

Mingazzini, in his work on Periplaneta orientalis, stated also that these modified cells were glandular in function and probably secreted an oily substance. So much did these cells resemble, both in appearance and position, those cells in Apis, Trigona, Bombus, Melepona, and Aphrophora, which were definitely known to secrete wax, that it occurred to the writer that possibly they might also perform this same function in the case of the cockroach. Tests were accordingly made to see if wax was present in these insects. A large number of the insects were captured and thrown into a bottle of warmed chloroform. After remaining in this liquid for ten minutes, they were strained out before the chloroform could have had time to have penetrated into the interior of the insect and attacked the fat in the fat cells. The chloroform was then filtered and divided into two portions. From one of these portions the chloroform was entirely evaporated on a water-bath. When the chloroform had been entirely driven off, a brownish liquid was left which possessed the characteristic cockroach odor and which, on cooling, solidified into a greasy appearing substance.

The next point was to find out whether this substance was grease or a wax. It is well-known that fatty oils, or fats treated with basic hydroxides, are decomposed into fatty acids and glycerol, this decomposition being hastened by using alcohol as a solvent for the alkali. Both the fatty acids and glycerol are

soluble in water, so that when water is added no precipitate would be formed. On the other hand, waxes, when similarly treated, yield smaller amounts of fatty acids and, instead of glycerol, give a large proportion of alcohol of the CnH_{2n+1} series, which is a solid body insoluble in water. Consequently, when water is added to these, a distinct precipitate is formed.

The greasy appearing material obtained from the cockroaches was treated in this way. A small amount of pure beeswax was similarly treated as a check. In both cases, after the two substances had been saponified and the liquid evaporated over a water-bath, a decided precipitate was obtained on the addition of water, more heavy in the case of beeswax, no doubt because more of the wax was present. It seemed, therefore, that the material secreted on the outside of the cockroach was wax.

In order to substantiate the preceding conclusion, a series of solubility tests were made with the second portion of the chloroform in which the roaches had been dropped. These tests were similar to those carried out by Hollande ('14) in his research on the oenocytes of insects, when he demonstrated that the crystals found in these bodies were wax crystals. In order to obtain wax for these tests a few drops of the chloroform was placed on a glass slide and then subjected to a current of air. By this means the chloroform was quickly evaporated, leaving a small residue of wax, appearing as a whitish spot on the slide. Slides thus prepared were immersed in the various reagents for periods varying from fifteen minutes to one-half hour. Observations were then made to see whether or not the material had been dissolved. Beeswax and wax from Pseudococcus citri dissolved in chloroform were used as checks. The results are as per table, page 572.

The saponification and solubility tests therefore clearly indicate that wax is present on the body of the cockroach. Therefore, because wax is present on the bodies of these insects and glands are found in their abdomens resembling glands found in other insects which are known to secrete wax, it seems conclusive that these modified hypodermal cells are true wax glands.

REAGENT	BEESWAX	COCKROACH MATERIAL	PSEUDOCOCCUS WAX
KOH sol	Insoluble	Insoluble	Insoluble
NaOH sol	Insoluble	Insoluble	Insoluble
NH_4OH	Insoluble	Insoluble	Insoluble
Alkaline soap. sol	Insoluble	Insoluble	Insoluble
Citric acid	Insoluble	Insoluble	Insoluble
Tartaric acid	Insoluble	Insoluble	Insoluble
Oxalic acid	Insoluble	Insoluble	Insoluble
Acetic acid	Insoluble	Insoluble	Insoluble
H_2SO_4	Insoluble	Insoluble	Insoluble
HNO_3	Insoluble	Insoluble	Insoluble
HCL	Insoluble	Insoluble	Insoluble
Cold absol. alcohol	Insoluble	Insoluble	Insoluble
Acetone	Insoluble	Insoluble	Insoluble
Olive oil	Insoluble	Insoluble	Insoluble
Cold alcoholic potash	Insoluble	Insoluble	Insoluble
Ether	Soluble	Soluble	Soluble
Chloroform	Soluble	Soluble	Soluble
Xylol	Soluble	Soluble	Soluble
H_2SO_4 + formol	No coloration	No coloration	No coloration
Sodium carbonate	Insoluble	Insoluble	Insoluble
Ammonium carbonate	Insoluble	Insoluble	Insoluble

DEVELOPMENT OF THE GLANDS

Mingazzini, in his work on Periplaneta orientalis, found that these gland cells were less developed in the nymphs than in the adult. In such immature stages he found that sections showed only a single layer of cells, between which were observed frequently large modified cells. In Blatta germanica these gland cells were first distinguished during the first instar when the nymphs are two days old (fig. 11). At that time some of the normal hypodermal cells become modified. The nuclei which are deeply stained and densely granular, gradually increase in size still retaining their densely granular appearance. There is also an increase in the amount of cytoplasm around these nuclei, with the result that the adjacent normal hypodermal cells are crowded so that their regular arrangement is somewhat broken. The growth in size of the nucleus and the increase of cytoplasm also cause these cells to project below the level of the normal

hypodermis, so that they stand out rather conspicuously. Finally, the cytoplasm becomes granular, the dense chromatin granules in the nucleus seem to assemble in large clumps, and the nucleolus, which up to this time could not be distinguished, becomes clearly visible. The transition from normal hypodermis to glandular cells can thus be readily observed in two-day-old nymphs. In the third instar the condition characteristic of the adult is attained, the cells being fully developed, and in some cases so closely packed together as to simulate the appearance of two or more layers.

FUNCTION OF THE WAX

The secretion of wax in insects serves various purposes—protection against enemies, cold, and moisture, a protection for eggs, an encasement for excreta, a lining for the larval burrow, etc. In the cockroach it probably serves as a protection against moisture, for they live in protected places where other enemies are unable to attack them. On the other hand, their presence in warm, damp places intimately associated with water would seem to indicate that the wax was secreted to protect them from this.

The fact that the wax possesses the characteristic 'roachy' odor suggested the idea that it might be a protection against enemies on account of its smell. However, an examination of beeswax and Pseudococcus wax showed that these also possessed characteristic odors. That of beeswax is well known. The odor from Pseudococcus was like mouldy wet leaves. That of the cockroach has already been described. From this it would seem that the wax secreted by each different family of insects had an odor 'sui generis.' The fact that the 'roachy' odor remains in roach-infested places for a considerable period of time after the roaches have been driven away or exterminated and that it is difficult to remove it from dishes and kitchen utensils may be accounted for by the fact that the wax remained adhering to these things over which they were accustomed to run.

The writer wishes to acknowledge his sincere thanks to Dr. W. A. Riley, whose advice and criticism have been invaluable and whose encouragement has made this work possible.

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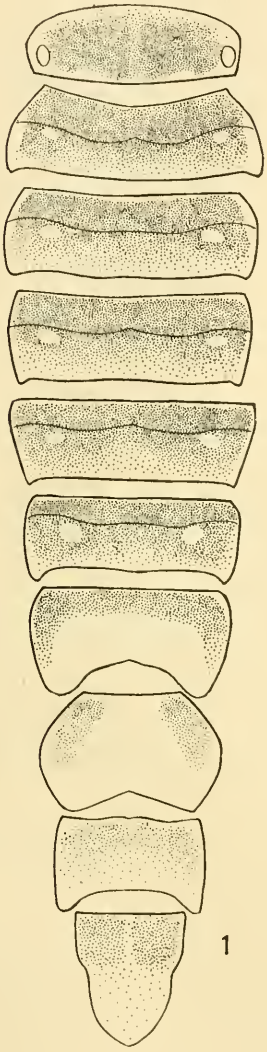
PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Dorsal abdominal surface of male, showing distribution of the wax glands.
2 Ventral abdominal surface of male, showing distribution of the wax glands.



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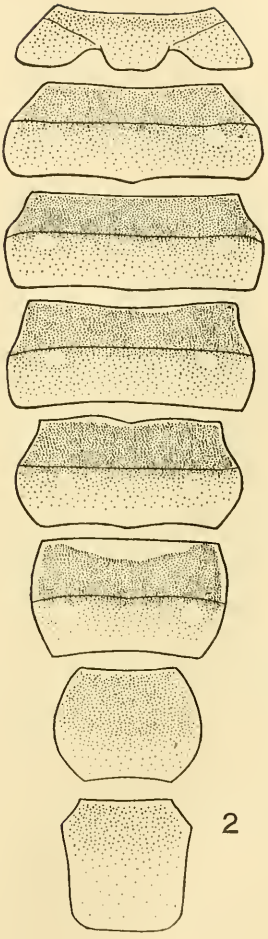

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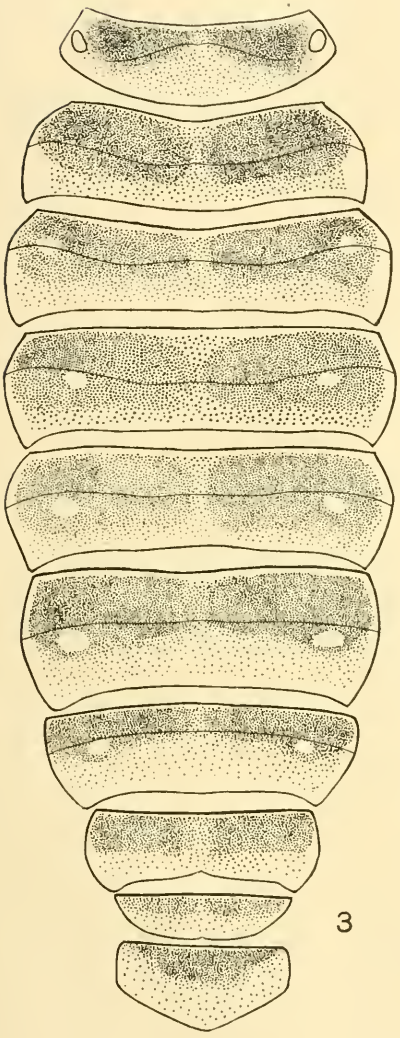
PLATE 2

EXPLANATION OF FIGURES

3 Dorsal abdominal surface of female, showing distribution of the wax glands.

4 Ventral abdominal surface of female, showing distribution of the wax glands.



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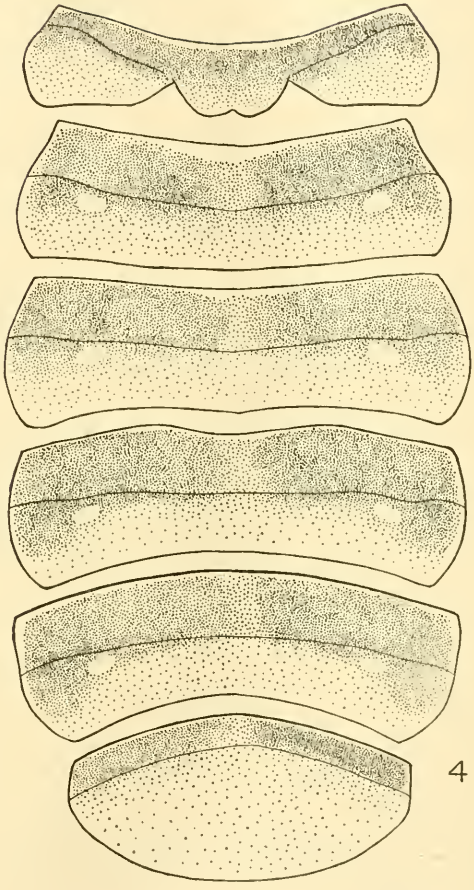

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PLATE 3

EXPLANATION OF FIGURES

5 Surface view of the wax glands (focus below the non-glandular hypodermis). × 600.

6 Surface view of normal hypodermis of the intersegmental membrane. × 600.

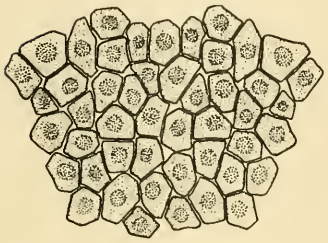
7 Surface view of cuticula overlying the glandular areas showing pores. × 600.

8 Longitudinal section through a single intersegmental area on the dorsal surface of the female, lateral to the middle line, showing the appearance and location of the wax glands. *i*, intersegmental membrane; *c*, cuticula; *h*, hypodermis; *g*, wax glands; *b*, basement membrane. × 160.

9 Longitutinal section through a single intersegmental area on the ventral surface of the male, showing appearance and location of the wax glands. *a*, muscles; *c*, wax glands, *b*, basement membrane; *d*, intersegmental membrane; *e*, hypodermis; *f*, cuticula. × 160.

10 Portion of the body wall in the region of the wax glands, showing detailed structure. *n*, non-glandular hypodermis; *p*, pores; *c*, cuticula; *v*, vacuole; *r*, basement membrane. (No. 10 ocular, 1.8-mm. oil immersion.)

11 Longitudinal section through a single intersegmental area of a two-day-old nymph, showing the development of the gland. *u*, hypodermis; *t*, transforming hypodermal cell; *s*, fully-transformed cell. × 600.



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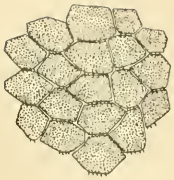

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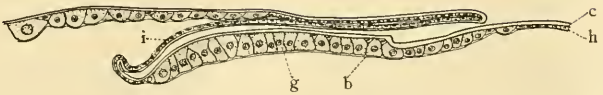

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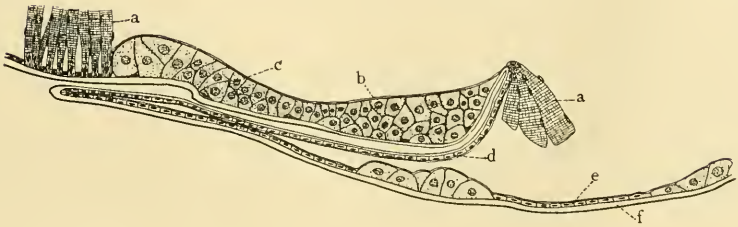

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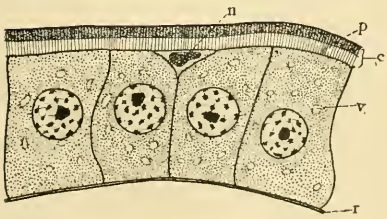

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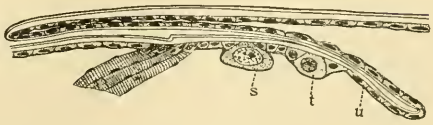

11

Resumido por el autor, Charles Eugene Johnson.

El origen del cuerpo último-branquial y su relación con la quinta bolsa visceral en las aves.

Las observaciones efectuadas por el autor en los embriones de gallina de agua (Fulica americana), gallinula, golondrina de mar, y gallina, demuestran que la verdadera quinta bolsa visceral de las aves es un divertículo diferente del que da origen al cuerpo último-branquial. Este cuerpo, al contrario de lo que se ha aceptado hasta ahora, no se forma a expensas de la quinta bolsa sino a expensas de un divertículo que se desarrolla medialmente a dicha bolsa. Observaciones efectuadas en la tortuga Chelydra serpentina y la comparación de los resultados mencionados con los obtenidos en otros reptiles, demuestran que la quinta bolsa y el divertículo último-branquial de las aves son homólogos con las estructuras correspondientes de los reptiles.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
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THE ORIGIN OF THE ULTIMOBRANCHIAL BODY AND ITS RELATION TO THE FIFTH POUCH IN BIRDS

CHARLES EUGENE JOHNSON

Department of Animal Biology, University of Minnesota

TWO TEXT FIGURES AND TWO PLATES

The authority on which the accounts in text-books of embryology of the origin of the ultimobranchial body in birds are based is the work of Verdun ('98) on the chick. According to him, the ultimobranchial body arises from a diverticulum of the pharyngeal wall just behind the fourth visceral pouch. He calls this diverticulum the fifth visceral pouch, and he found it first in embryos of 124 hours. It was not present in embryos of 96 hours, he states, and at this stage the fourth pouch also was lacking. For an embryo of 148 hours he illustrates it as a caudally directed sac-like outpouching of approximately the same size as the fourth visceral pouch itself, at the base of which it opens into the pharynx through a wide mouth.

In a survey of the literature on the subject in the chick one finds the accounts of the development of the ultimobranchial body and its relations to the fifth pouch rather indefinite and conflicting. Kastschenko ('87) found the so-called fifth pouch in a chick embryo of four days (illustrated in his figures 11 and 14, plate 19). Again in an embryo of six days he recognized a large 'bulbose Anschwellung' connected on one hand with the fourth visceral pouch and on the other with the pharynx, which "nichts anderes als die fünfte Schlundtasche darstellt." This fifth pouch, he states, has remained connected with the fourth pouch during the elongation of the latter and retains the connection also at a later period, after the two thymus anlagen have been separated from the pharyngeal wall. He refers to de Meu-

ron's ('86) statement that this fifth pouch becomes detached from the pharynx and also from the fourth pouch in embryos of six days, but remarks that in his own material he did not observe the separation from the fourth pouch, although he examined a number of embryos of that age.

Mall ('87) derived the ultimobranchial body ('corpus y') from what was called by him the 'fossa subbranchialis' and which from his description differs in no way from the fifth pouch of Kastschenko and other authors. Mall mentions this fossa subbranchialis first, and illustrates it clearly, in the description of the visceral pouches of an embryo of three days, seven hours.

Liessner ('88) recognized the fifth pouch in embryos of 84 hours. At 120 hours, he states, the fifth pouches reach their maximum development and at 126 hours they have completely disappeared. The ultimobranchial body is not mentioned by Liessner, and the meaning of his statement that the fifth pouches disappear is not clear.

In none of the accounts of the development of the visceral pouches and the ultimobranchial body in the chick is there any reference to more than one diverticulum associated with the fourth visceral pouch, and all authors, with the exception of Mall, who describes the 'fossa subbranchialis' as an outpouching from the ventral aboral region of the fourth pouch, call this diverticulum the fifth visceral pouch.

In the duck and the house-sparrow (Passer domesticus) Kallius ('05) demonstrated the presence of a well-developed fifth visceral pouch, but the ultimobranchial body is not discussed by this author. On the other hand, Helgessen ('13) for the house-sparrow and Hamilton ('13) for the duck make no mention of a fifth visceral pouch, but derive the ultimobranchial body from the fourth pouch.

Rabl ('07) was the first to demonstrate the existence of two distinct diverticula in connection with the posterior wall of the fourth pouch, one of which represents the true fifth pouch and the other the anlage of the ultimobranchial body. His observations were made on the duck. The ultimobranchial diverticu-

lum was interpreted by Rabl to represent a true sixth visceral pouch in birds.

My own observations ('17) have shown that two diverticula corresponding to those in the duck are developed in the pied-billed grebe. It is the purpose of the present paper to report the development of two such evaginations also in species representing two additional families of wild birds and in the chick.

Coot and gallinule. These two species present practically identical conditions. They show particularly well the relations of the fifth pouch and the ultimobranchial diverticulum in birds. Figure 3 represents the structures in this region of a gallinule (Gallinula galeata galeata Licht.) of approximately four and one-half days. The fourth pouch has a relatively long dorsoventral axis. The fifth pouch forms a narrower parallel diverticulum which is about three-fourths as long dorsoventrally as the fourth, is directed slightly more caudally, and at no point touches the ectoderm. The two pouches are at the end of a rather elongate common diverticulum of the pharynx which, because of an approximation of the two pouches in width, has the appearance in sections of being divided distally into twin lobes, very similar to the condition in the duck as described by Rabl. The fifth pouch of the left side is distinctly longer and broader than that of the right. The fifth aortic arches are complete. That of the left side lies in the angle between the fourth and the fifth pouch, i.e., in the fifth visceral arch, but the one on the right courses along the lateral border of the fifth pouch. The ultimobranchial diverticulum is just beginning to appear on the left side of the body as a slight bulging of the common pharyngeal diverticulum, medial to the fifth pouch.

Figure 5 represents the parts concerned in a coot (Fulica americana Gmel.) of about six days. The fifth pouch, though still conspicuous, is much reduced as compared with that of the four and one-half-day gallinule. The fifth aortic arch has in greater part disappeared, its dorsal end alone persisting as a short branch of the sixth. On the right side the diverticulum of the ultimobranchial body is not yet discernible, but on the left

it is well differentiated and is situated entirely mediad of the fifth pouch, forming an evagination of the common pharyngeal diverticulum. This common pharyngeal diverticulum, as a result apparently of its further outgrowth and the consequent narrowing of its connection with the pharynx, together with the gradual reduction of the fifth pouch, now has more the appearance of being the fourth pouch proper, with the fifth pouch and the ultimobranchial diverticulum forming merely secondary outgrowths from its wall.

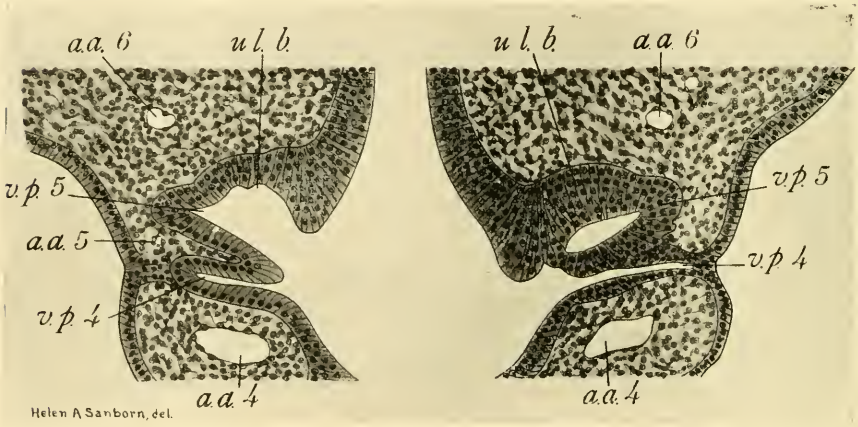

Fig. 1 Horizontal section through the region of the fourth and fifth visceral pouches and the ultimobranchial diverticulum of an embryo Forster's tern of four and one-half days. *A.a.4,5,6*, fourth, fifth and sixth aortic arches; *v.p.4,5*, fourth and fifth visceral pouches; *ul.b.*, ultimobranchial body. × 112.

Forster's tern. The youngest embryo of this tern (Sterna forsteri Nutt.) in my collection is one of about four and one-half days. In this specimen the fifth pouch is well developed (fig. 1). At its junction with the pharyngeal wall is a second clearly defined diverticulum, present on both sides of the embryo. This is the anlage of the ultimobranchial body. It differs from the corresponding diverticulum in the coot and the gallinule, in making its appearance somewhat earlier and in being more closely associated with the fifth pouch; but it agrees in these respects with the conditions presented by the four-day duck (Rabl).

The only other embryo of this species in my possession of sufficiently early developmental stage to show remains of the fifth visceral pouch is a specimen of approximately six and one-half days. The pouch (fig. 6) is here on the verge of disappearing, but can be definitely identified in the sections. The ultimobranchial diverticulum is a relatively large outgrowth of globular form, sharply differentiated on both sides of the embryo, but that on the left considerably larger, as in the other species considered.

Chick. In the chick, judging from the available material only, the early steps in the development of the fifth pouch and the ultimobranchial diverticulum are not so clear as in the preceding species. In an 80-hour specimen there is a well-defined diverticulum having a form and relationship quite similar to the fifth pouch of the coot and the gallinule, in the early stages of the pouch in these forms. In a 90-hour embryo the corresponding diverticulum is relatively very large, appearing actually larger than the fourth pouch itself, and is thicker-walled. In two 100-hour embryos this evagination appears to have reached its greatest development. It agrees in the main with the so-called fifth pouch as that has usually been described for the chick, forming a deep sac-like out-growth behind the fourth pouch, its blind end directed caudad and slightly laterad towards the ectoderm. It has a broader connection with the common pharyngeal diverticulum than had the fifth pouch in either the coot or the gallinule and is of more rounded form. It is also situated relatively nearer the pharyngeal wall, a situation which may have been brought about by a further lateral growth of the fourth pouch. In one of these specimens the so-called fifth pouch shows, on each side of the body, a slight indication of division into a lateral and a medial lobe, similar to the division shown in the four and one-half day tern, but the evidence in the present case is not so clear. One gains the distinct impression, however, in examining this so-called fifth pouch in the 100-hour and 96-hour embryos, that it represents something more than merely the fifth pouch, such as it is characterized in the other species discussed. From analogy with the relations of the fifth

pouch and the ultimobranchial diverticulum in the tern it is possible that in the chick the two diverticula are coalesced in their early stages and only at a later period do they become distinguishable from each other, as the ultimobranchial body continues development while the fifth pouch becomes regressive. On the other hand is the possibility that in a more extensive series of chick embryos a condition similar to that in the tern may be found.

The next developmental stage at hand is represented by two embryos of five and one-half days. In both of these the ultimobranchial diverticulum is distinctly differentiated. Figure 4 is a wax reconstruction of the caudal pharyngeal diverticula of one of the embryos, made at a magnification of 200 diameters. The fourth visceral pouch was in close contact with the ectoderm. On the posterior wall of this pouch, where it joins the pharynx, are two distinct outpouchings. The lateral and smaller one clearly corresponds to the fifth pouch as it was represented in the preceding birds; the larger medial one is the ultimobranchial diverticulum. While the fifth pouch might here be interpreted as a secondary evagination from the fourth pouch, the ultimobranchial diverticulum is more clearly an outgrowth from the pharynx proper, and at this stage it opens into the pharyngeal cavity separately from the common mouth of the fourth and fifth pouches. The diverticulum as a whole is situated more ventrally than the fifth pouch. As in the other birds investigated, both the ultimobranchial diverticulum and the fifth pouch are better developed on the left side than on the right. Text figure 2 represents a transverse section through the middle of the fifth pouch of this embryo. The section cuts the ultimobranchial diverticulum at its junction with the pharyngeal wall, leaving the greater part of it posterior to the plane of the section.

In this embryo the fifth aortic arch could not be found on the left side, but on the right it was clearly represented by a short channel along the lateral side of the sixth arch, opposite the shallow groove formed between the fourth and fifth visceral pouches.

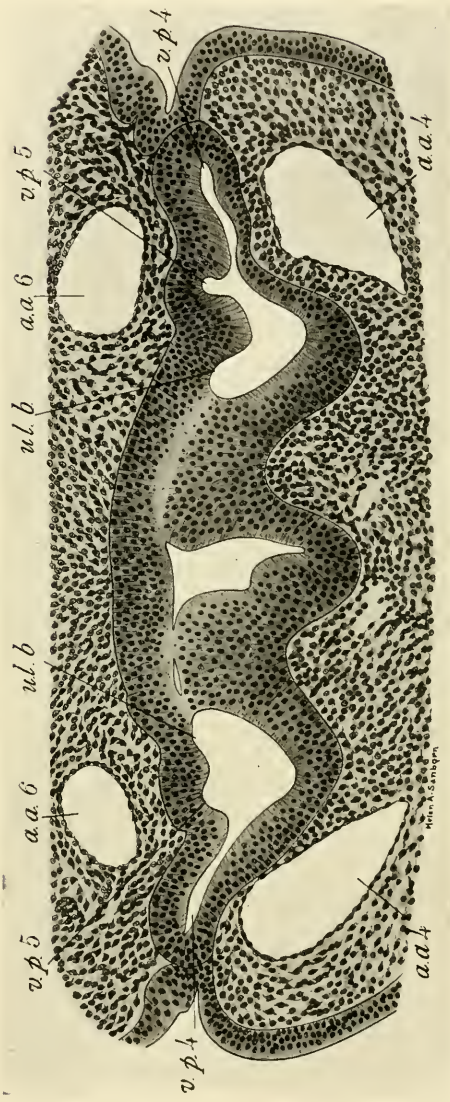

Fig. 2 Transverse section through the region of the fourth and fifth visceral pouches and the ultimobranchial diverticulum of a chick embryo of five and one-half days. *A.a.4,6*, fourth and sixth aortic arches; *v.p.4,5*, fourth and fifth visceral pouches; *ul.b.*, ultimobranchial body. × 100.

DISCUSSION

The evidence presented above shows that in birds a fifth visceral pouch is developed which is a different diverticulum from that which gives rise to the ultimobranchial body. This fifth pouch and the ultimobranchial diverticulum are strictly comparable to and homologous with the fifth pouch and the ultimobranchial diverticulum of reptiles.

For the lizards, Peter ('00) and Saint-Remy et Prenant ('03–'04) in more recent years have demonstrated the presence of five visceral pouches, exclusive of the diverticulum giving rise to the ultimobranchial body. In Anguis fragilis, Lacerta viridis, and L. agilis, Saint-Remy et Prenant found that a relatively small fifth pouch is developed, which, however, soon disappears without leaving any trace. The left ultimobranchial body alone undergoes progressive development while the right usually vanishes, as in birds.

In the snakes these same authors also found five visceral pouches, but the fifth pouch here gives rise to a portion of the thymus and to a parathyreoid body. The ultimobranchial body persists on both sides.

In the turtles, van Bemmelen ('93) found that in Chelonia viridis (mydas) the fourth and fifth pouches are developed simultaneously with the ultimobranchial diverticulum from a lateral 'blinddarmförmigen Falte' at the posterior end of the pharynx. These pouches and the ultimobranchial anlage soon become pinched off from the pharynx and form a complex of three united vesicles.

In embryos of Chelydra serpentina I find that the structures in question arise in a similar way to those of Chelonia viridis. In an embryo of 6 mm. the fourth and fifth pouches and the ultimobranchial diverticulum can be recognized in the form of three secondary outpouchings from a relatively large globular primary evagination of the pharynx. By the 9-mm. stage a deep constriction has appeared extending dorsoventrally in the transverse plane and separating the fourth pouch on the one hand from the fifth pouch and the ultimobranchial anlage on the other;

but the two divisions thus formed are united at the base and connect with the pharynx through a common passage. The fifth pouch is a small elongate diverticulum arising from the lateral side of the ultimobranchial outpouching, i.e., where this outpouching joins the common pharyngeal stalk. It extends parallel with the fourth pouch and comes into close contact with the ectoderm. The right pouch is considerably larger than the left. On each side the pouch projects through the vascular ring formed by the fifth and sixth aortic arches, the fifth arch being very short and joining the sixth immediately above and below the pouch. The disparity in size between the fourth and the fifth pouch in the turtle at this stage is greater than that between the corresponding pouches in the four and one-half day gallinule. The ultimobranchial diverticulum on the other hand is relatively very much larger. Its place relations, however, are practically identical with those of the ultimobranchial diverticulum in the birds described.

The formation of the fifth visceral pouch and the ultimobranchial diverticulum in birds and in reptiles is essentially the same. In the reptiles, as represented by the turtles, these evaginations are differentiated simultaneously with the fourth visceral pouch from a common pharyngeal outpouching. This may possibly be explained by the relatively large pharyngeal area concerned with the formation of the ultimobranchial body. In the birds the fourth and fifth pouches appear first. The ultimobranchial body is somewhat retarded and its anlage involves a relatively smaller area. The fifth pouch of birds, like that of both lizards and turtles, is small and unimportant; apparently it only rarely gives rise to thymus tissue. The variations in its developmental details are probably governed by those of the greater and more important diverticula of the fourth pouch and the ultimobranchial body.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

3 Posteroventral view of a wax reconstruction of the caudal portion of the pharynx and the associated aortic arches of an embryo gallinule of four and one-half days. × 150.

4 Posteroventral view of the caudal portion of the pharynx of a chick embryo of five and one-half days. × 150. *A.a.4,5,6*, fourth, fifth and sixth aortic arches; *A.p.*, pulmonary artery; *A.v.*, ventral aorta; *Oes.*, oesophagus; *Tr.*, trachea; *Ul.b.*, ultimobranchial body; *V.p.4,5*, fourth and fifth visceral pouches.

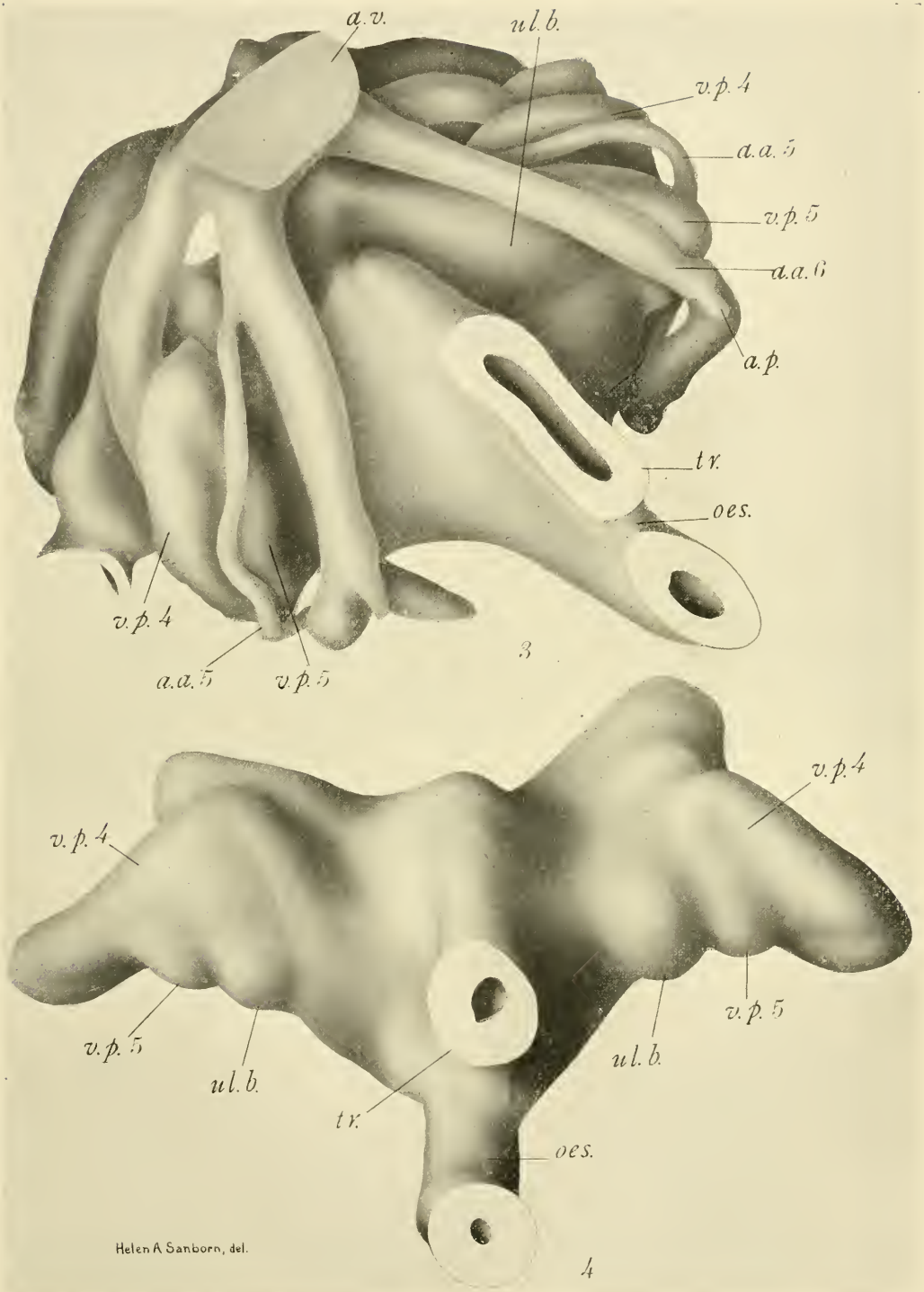
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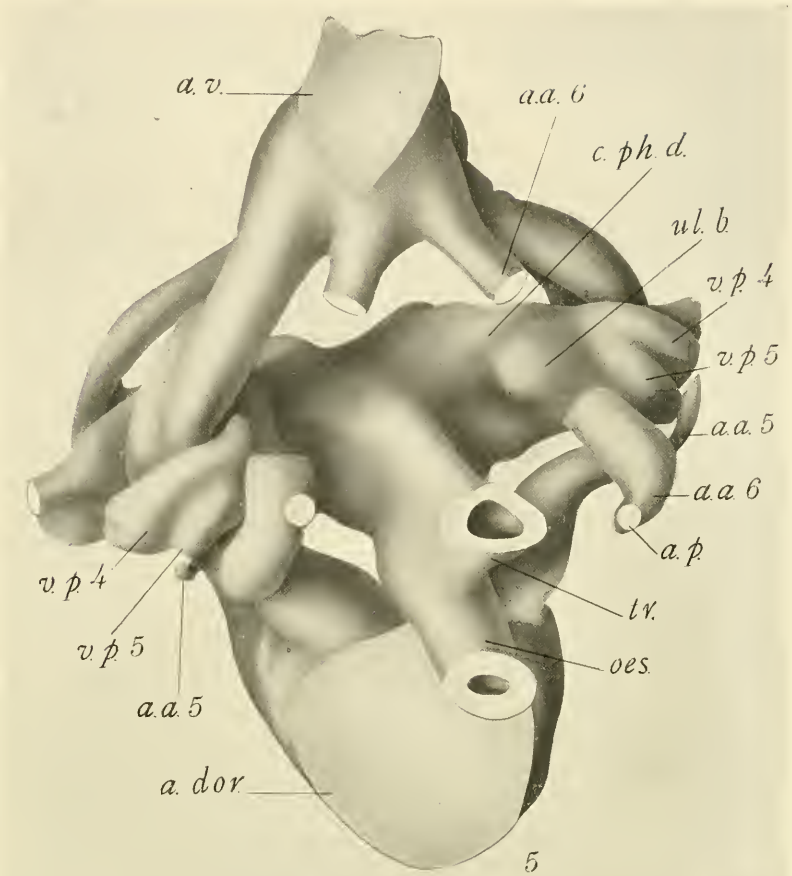
PLATE 2

EXPLANATION OF FIGURES

5 Posteroventral view of a wax reconstruction of the caudal portion of the pharynx and associated aortic arches of an embryo coot of six days. × 107.

6 Posterodorsal view of a wax reconstruction of the caudal portion of the pharynx of an embryo Forster's tern of six and one-half days. × 107. *A.a.3,5,6,* fourth, fifth, and sixth aortic arches; *A.dor.*, dorsal aorta; *A.p.*, pulmonary artery; *A.v.*, ventral aorta; *C.ph.d.*, common pharyngeal diverticulum; *Oes.*, oesophagus; *Tr.*, trachea; *Ul.b.*, ultimobranchial body; *V.p.4,5,* fourth and fifth visceral pouches.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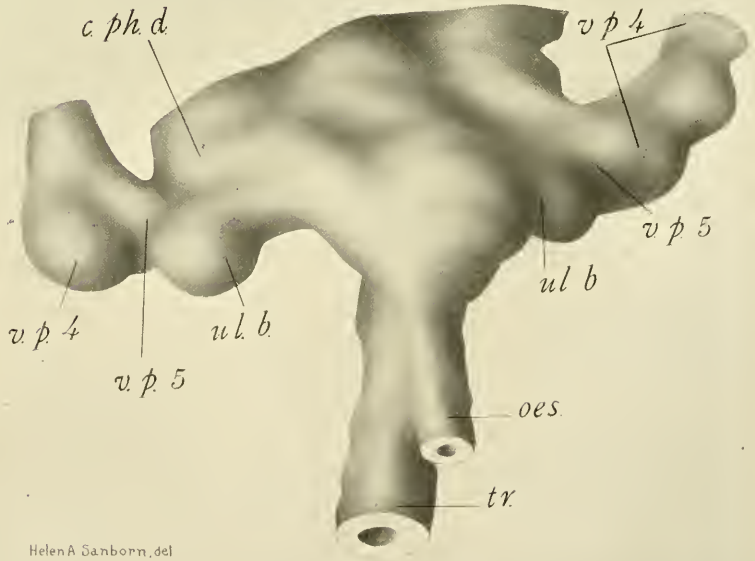
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Resumido por el autor, R. W. Shufeldt.

Notas sobre la osteología y otros puntos de la morfología del jóven del hoatzin (Opisthocomus cristatus).

El material para el estudio anatómico de Opisthocomus cristatus ha sido susministrado por Mr. Robert Cushman Murphy ('14–'15); consistia en individuos jóvenes y subadultos capturados por Mr. George Cherrie en la Guyana. Todas las figuras (cuatro láminas) han sido dibujadas por el autor; representan las partes externas y el esqueleto, y son reproducción de fotografias hechas por el autor. Se describe por vez primera el esqueleto del pollo y subadulto, comparando incidentalmente sus caracteres con los correspondientes del esqueleto del adulto. Tambien se revisa una gran parte de la literatura referente a este punto. Después de describir con detalle el cráneo, el autor hace notar que hay probablemente cuarenta y cuatro vértebras en la columna vertebral, y que la morfología de la pelvis coincide notablemente con la que presenta dicho hueso en la gallina. Todo el esternón, a excepción de una pequeña porción anterior de la quilla sumamente rudimentaria, permanece cartilaginoso durante largo tiempo; la forma del hueso en el adulto se conoce hace largo tiempo. También se describe con detalle el desarrollo de los huesos de los miembros, considerando con especial atención el enorme tamaño de los piés en estos animales jóvenes, en comparación con el tamaño general de las restantes partes del cuerpo. El tegumento es grueso y coriáceo; la reticulación y escutelación de las podotecas se ven claramente puesto que son reproducciones de fotografias. Tambien se presta atención a la pterilosis, glándula uropigia y otros puntos de la anatomía topográfica de este ave.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, DECEMBER 2

NOTES ON THE OSTEOLOGY OF THE YOUNG OF THE HOATZIN (OPISTHOCOMUS CRISTATUS) AND OTHER POINTS ON ITS MORPHOLOGY

R. W. SHUFELDT

EIGHT FIGURES (FOUR PLATES)

In the winter of 1914–15, I received from Mr. Robert Cushman Murphy several specimens of the subadult and young of Opisthocomus cristatus. My impression is they were collected in Guiana by Mr. George Cherrie though I may be mistaken in regard to this. Dr. James E. Benedict, of the United States National Museum, had Mr. Scollick, the skillful preparateur of that institution, prepare the skeletons of two of these specimens for my use in descriptive work (figs. 1 and 2). Upon this account I presented the entire lot to the National Museum in February, 1914. (Mus. No. 223903.)

There is considerable literature on the hoatzin, which dates back to the writings of Müller and of Gmelin, the latter passing the bird into the genus Phasianus of Linnaeus, while Buffon considered it to be a curassow. Illiger created the genus Opisthocomus for it in 1811, and, twenty-six years later, L'Herminier gave a fairly good account of its anatomy.

Figure 1 is from a subadult specimen, taken in the pin-feather, just before it is able to fly; the bird was probably double the age of the chick whose skeleton is shown in figure 2. In the latter, almost the entire skeleton is still in cartilage, although minute ossific centers can be detected in the basi-occipital of the skull, but not distinctly in any other bone. However, even at this age the main osteological characters of the species are quite apparent.

Superficially, the skull, as a whole, reminds one of the skull of the chick of Gallus; though upon direct comparison, character for character, the resemblance is soon dispelled. As in the adult,

the culmen of the superior mandible has a uniform curve from the craniofacial hinge to the rather sharp apex; in fact, the upper bill as a whole slopes away to its tip in a gentle forward and downward direction. Laterally, the small narial aperture is rather irregular in outline and is situated about half way between the anterior margin of the orbit and the distal apex of the beak.

The V-shaped mandible is nearly uniform in depth from its articulation to its symphysis, which latter is truncate from the terminal point, downwards and backwards.

Either orbit is subcircular in outline and possesses a very sharp margin for the entire arc of its superior periphery. Anteriorly, the pars plana is complete. Even at this early stage the interorbital septum is entire for its whole extent; the nerve foramina on its posterior joining with the anterior wall of the brain-case are small, being only of sufficient size to admit of the passage of the nerves through them. Even the floor of this orbit is more or less complete, made so by the close articulation of the palatine of the same side, and, in part, by the breadth of the corresponding quadrate bone and also, to some extent, the pterygoid.

Externally, the vault of the cranium is rounded and smooth, being but slightly depressed between the orbits mesially and in front; and the nasals extend well backwards, their posterior apices in the middle coming in contact opposite the center of the orbits.

The basis cranii is in the horizontal plane and the condyle is of considerable size.

In the vertebral column there are seventeen cervical vertebrae in the skeleton of the neck, and of these the last two, the sixteenth and seventeenth, support a pair of free ribs. These ribs lack unciform processes, while those of the three dorsal pairs of ribs are peculiar in that they are elongate, nonprojecting backwards, and more or less adpressed anteriorly to the posterior margin of the rib in all cases. This is also the case with the anterior pair of pelvic ribs.

The dorsal vertebrae are closely articulated with each other, the neural spines being low, and, in their present cartilaginous state, appear to be almost blended with each other. No haemal

spines have developed on any of the vertebrae of the column; and, as a matter of fact, their processes are very simple and much reduced (fig. 1).

The vertebral as well as the sternal ribs are broad and flat; there are five pairs of the latter, and each pair articulates with the sternum independently, even the two pairs that meet the pelvic ribs, which latter possess epipleural appendages only in the case of the anterior pair.

In the articulated, cartilaginous skeleton it is not easy to count correctly the number of vertebrae in the column between the last dorsal and first caudal ones. However, I have counted them over several times, and there appear to be fifteen of these; that is, five beneath the anterior part of the ilia and ten posterior to them, which latter can be counted with great certainty. There are six caudal vertebrae and three more in the pygostyle, which makes nine of these segments in the skeleton of the tail. Judging, then, as best I can, there appear to be 44 vertebrae in the spine of Opisthocomus cristatus; but in order to be certain of this, I should like to see the skeleton of a specimen about a month older than either of these.

When the bird is as old as the one which furnished the skeleton shown in figure 1 of the present contribution, the ischia of the pelvis exhibit some little advance in ossification; there is also some bone formed in either ilium, in a strip of some width running along the outer moiety of the preacetabular portion, as far back as the middle point over the elliptical ischiadic foramen. The pubic style, which extends far beyond the ischium on either side, has also commenced to ossify along its anterior part. All the posterior parts of these pelvic bones are still in cartilage; and upon the whole, this pelvis possesses much the same appearance, in matters of form and development, as it does in the pullet of the common fowl.

With respect to the shoulder-girdle or pectoral arch, the os furculum is entirely cartilaginous at the stage of development shown in figure 1; while a coracoid as well as a scapula have ossified to a very considerable extent, though the extremities of these bones are still in cartilage. A scapula promises to be broad and short as well as considerably curved.

Curiously enough, I find the entire sternum, all to a small anterior portion of the extremely rudimentary keel, still performed in cartilage. This is thick and substantial; and, as the morphology of this bone, as it appears in the adult bird, has long been known, it requires no further description here. Its form on lateral view, in the subadult individual, is well shown in figure 1.

The skeleton of the limbs. All the long bones, including the phalanges of pes and manus, are, in the pullet, ossified, so far as their shafts are concerned, the proximal and distal extremities being more or less still in cartilage. These bones are similarly ossified in the chick; but the process has not proceeded to the same extent nor is the bony tissue so dense. In fact, it is what we may expect to find in an earlier stage of the process (figs. 1 and 2).

In the humerus, the radial crest is scarcely at all developed, and the bone as a whole exhibits, to quite a marked degree, the sigmoid curve from head to distal extremity, where the trochleae are still in cartilage.

The antibrachium is somewhat shorter than the arm, and of the two bones only the ulna exhibits any degree of curvature—even in its case it is not so very great.

Ulnare and radiale of the carpus are only in cartilage, while the three long bones composing the metacarpus are as yet separate—the one for the pollex digit being short and rather thick and attached parallel to the stout and very straight shaft of the index metacarpal. The medium metacarpal is slender, nearly of uniform caliber, and very much bowed, the concavity being toward the index metacarpal.

There is nothing peculiar about the terminal digits and claws, of which latter there are two, as shown in figure 1 of the present article. The medius digit or phalangeal joint is small and triangular in outline, being without a terminal claw.

In the pelvic limb (fig. 1), ossification of the several bones composing it has proceeded about as far as in the bones of the wing. The femur is but slightly bowed anteroposteriorly and promises to be stout and strong, as indeed all the bones of this limb are. Even in the pullet I fail to find any bony patella,

though the tendon in which it should occur is thick and broad. The tibiotarsus is bulky and thick, being much enlarged at its extremity—the distal, cartilaginous condylar portion being conspicuously extensive.

Very early in the development in the chick the three tarsal bones, which eventually fuse to form the tarsometatarsus, unite at the juncture of the middle and lower thirds of the shaft, the fusion being still further advanced in the pullet (fig. 1).

One of the most striking characters in the skeleton of the young Opisthocomus is the enormous size of the feet, as compared with the remaining proportions of the bird itself. This feature is, of course, by no means lost in the fowl as we find it in nature; but then the discrepancy does not seem to be so great on account of the presence of the plumage, which lends the appearance of greater bulk to the body. (Compare figs. 1 and 2 with 4 and 7.)

The phalanges are stout and relatively long; the ungual joints large and strong, and to some extent curved, especially the claw of the hallux. As in so many birds, the arrangement of the joints of the pedal digits is 2, 3, 4, to first, second, third, and fourth toes, respectively.

Transversely attached to the posterior border of the summit of the tarsometatarsus, just anterior to the short, single-grooved, cartilaginous hypotarsus, there is a tough, curved piece of cartilage which, in life, fits closely in between the condyles of the tibiotarsus. It appears as though it might, to some little extent, ossify later in life, as it does in certain gallinaceous birds—a group to which the hoatzin is, in a way, related, as its morphology seems to indicate. This piece of cartilage is of some considerable size even in the chick or nestling.

At the nestling stage, the integuments of this species are thick and tough, and these characters seem to be, to some extent, enhanced as the bird grows, for I find them to be still thicker and tougher in the pullet.

Figures 3 to 8, inclusive, give several of the characters of the superficial anatomy of the young of the hoatzin at the two stages of its existence here being considered. These show the reticula-

tion and scutellation of the podothecae, which heretofore have not been figured direct from photographs of the parts in question. These figures also show, with great accuracy, the relative proportions of the external features of the species.

It is well known that the big claws of pollex and index digits of the hand are more or less functional in this bird, and a number of naturalists have described the habit the young bird has of assisting itself, when 'climbing' through the twigs of the trees near the nest, by means of these clawed fingers; they are seen pretty well in figures 3, 4, and 8.

Upon examining the skin (alcoholic specimen) of the subadult specimen at hand, I find that the pterylosis can be studied with more or less satisfaction. The feathering upon the sides of the head and throat is very sparse, and there is a naked area around the eye, especially below the lower eyelid. The crest for the most part is median, though it spreads slightly upon either hand towards the side of the head by feathers greatly reduced in size.

In the cervical region the pterylosis is strong and continuous, with a total absence of naked spaces at the sides of the neck. Anteriorly, the feathering of the lower cervical area is carried down upon either side, to form rather broad, though not long, ventral pterylae—each one of which, below the thorax, is carried down to the vent as a narrow line of pretty strong feathers.

At the root of the neck dorsally, the pterylosis divides, to form strong, narrow humeral tracts, while in the median line the spinal tract is weak and narrow, particularly distally, where it is carried down as far as the oil-gland. At the root of the neck this spinal tract appears to be double; but the branches are very close together, and, upon proceeding forward, run together and are lost among the feathers forming the pterylosis at the base of the cervical region behind.

The crural pterylae are but faintly marked and the oil-gland is feathered. Feather-sheaths (pin-feathers) of the wings and tail are large and strong, the latter being imperfect in the specimen at hand, while I count nineteen remiges in the former, of which ten occur upon the pinion.

On the apteria, the down feathers are pretty well distributed over the body and limbs, and are well developed, being more or less abundant, particularly upon the sides of the body and upon the thighs. The free margins of the wings are also abundantly feathered from the thorax to the carpus of wither brachium.

This is as much as these young specimens show of the pterylosis of Opisthocomus cristatus; but it foreshadows what we may reasonably expect to find in the adult bird, which appears, in the main, to have been more or less correctly described by Nitzsch in his great classic on Pterylography. It may be well further to note that C. J. Sundevall ('73) describes a specimen which he casually examined; he came to the conclusion that the vinculum of the foot was absent, and consequently Opisthocomus must have passerine affinities. Garrod ('79), however, soon corrected this, and not only found the vinculum present, but of large size.

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PLATES

PLATE 1

(Reproductions of photographs made direct from the specimens by the author.)

EXPLANATION OF FIGURE

1 Left lateral view of the skeleton of a subadult specimen of the hoatzin (Opisthocomus cristatus); two-thirds natural size. From Mr. Robert Cushman Murphy, and prepared at the United States National Museum, to which institution it was presented by the author. (No. 223903.)

PLATE 2

EXPLANATION OF FIGURES

2 Skeleton of a nestling hoatzin (Opisthocomus cristatus); two-thirds natural size. Same lot as the one shown in figure 1, with same history. Skull and leading cervical vertebrae in right oblique lateral view. Skeleton of trunk twisted over so as to give a ventral view of the thorax, pelvis, and skeleton of tail. Wings rotated over with skeleton of trunk. Pelvic limbs separated from skeleton by dividing bones of the leg at their middles, in order to present more instructive views of them.

3 Dorsal view of the trunk and pectoral limbs of a nestling hoatzin (Opisthocomus cristatus). Head viewed upon left oblique aspect, and the right pelvic limb seen upon its outer side. Same lot as the one shown in figure 1, with the same history. Figures 1 to 3 alcoholic specimens; two-thirds natural size.

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PLATE 3

EXPLANATION OF FIGURES

4 Right lateral view of a nestling hoatzin (Opisthocomus cristatus); wings raised. Alcoholic specimen; two-thirds natural size. Same lot and history as the specimens in plates 1 and 2.

5 Direct left lateral view of the head of a young hoatzin (Opisthocomus cristatus); two-thirds natural size. Alcoholic specimen. Same lot and history as those figured on plates 1 and 2.

6 Feet of a nestling hoatzin (Opisthocomus cristatus); two-thirds natural size. Alcoholic. Same lot and history as those figured on plates 1 and 2. These feet are the same as the ones shown in figure 4 of this plate, seen upon left lateral aspect instead of upon the right.

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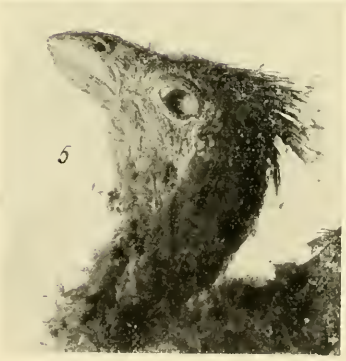
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PLATE 4

EXPLANATION OF FIGURES

7 Feet of a subadult hoatzin (Opisthocomus cristatus); two-thirds natural size. Same lot and history as the specimens figured on plates 1 to 3.

8 Right wing of a subadult hoatzin (Opisthocomus cristatus): two-thirds natural size. Alcoholic. Different specimen from the one shown in figure 7 of this plate. This wing is viewed directly from above, being spread out to show pin-feathers. Terminal claws of the digits seen among the feathers. Same history and lot as the specimens shown in figures 1 to 6 of plates 1 to 3.

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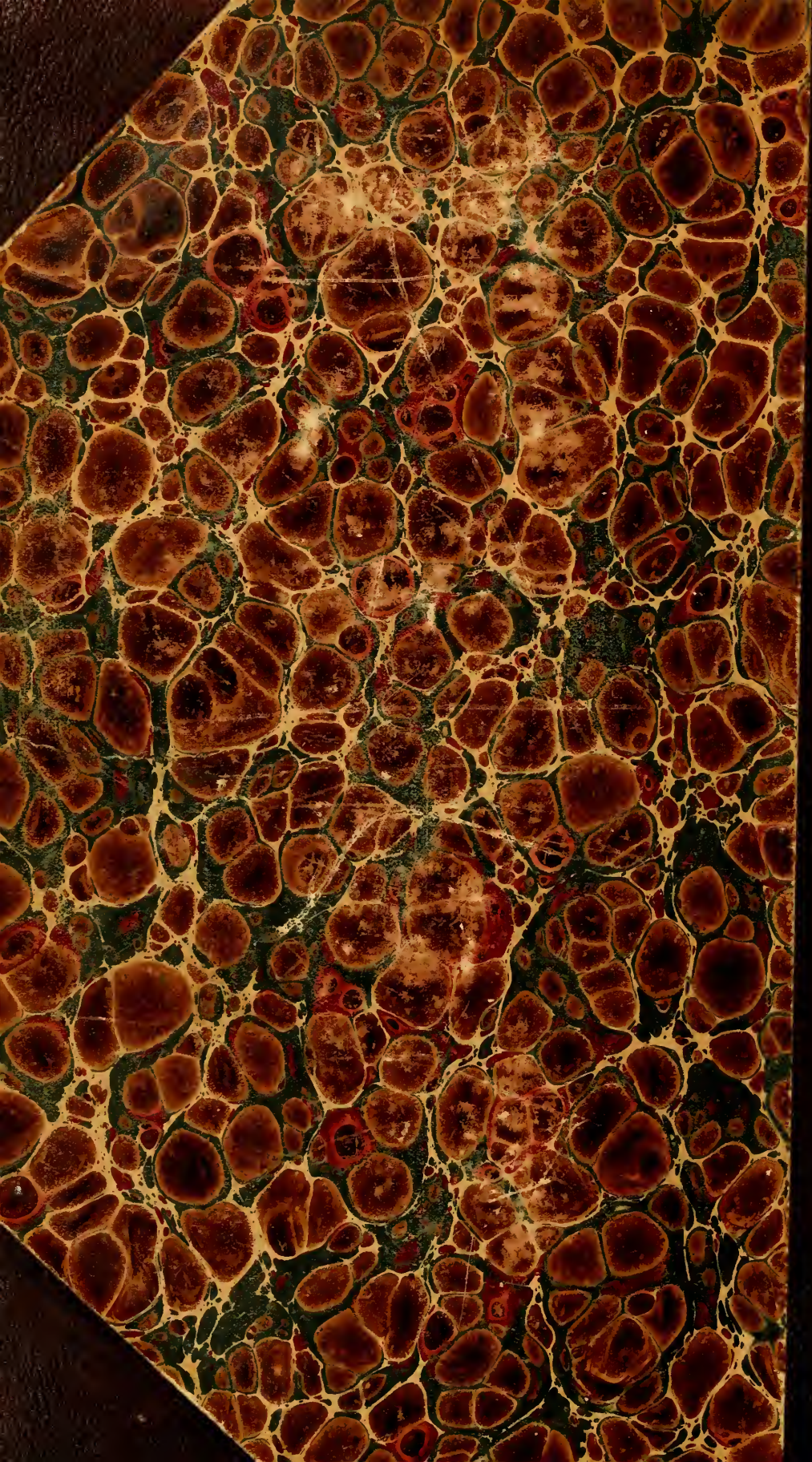
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